ANNUAL REVIEW OF PHARMACOLOGY

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PREFACE

The welcome accorded other *Annual Reviews* has encouraged us to see if this *Annual Review* might render similar service in pharmacology.

Where the world of pharmacology only a few years ago was largely academic and rather sparsely populated, it now extends to government and industry as well, and is getting settled, though not yet overcrowded. Publications have risen in accord with interest, and both outright pharmacological journals and pharmacological articles in other periodicals are greatly multiplied. Further, publications in pharmacology now go beyond the health professions. The impasse has clearly been reached, as in so many other fields of science, where one can no longer read all that one would like to, and finds reviews helpful. It is our intent that the reviews in the *Annual Review of Pharmacology* be critical and thus, we hope, helpful and that a summary of the progress of the current year or biennium be given in each general field of pharmacology.

By overlapping membership with the Editorial Board of *Pharmacological Reviews,* we hope to avoid duplication of topics, though in any event the purpose of broad annual reviews is different from that of reviews in depth of more limited fields.

We thank Professor J. Murray Luck for his general guidance and Miss Jeanine Ardourel, our editorial assistant, for her skill and devotion.

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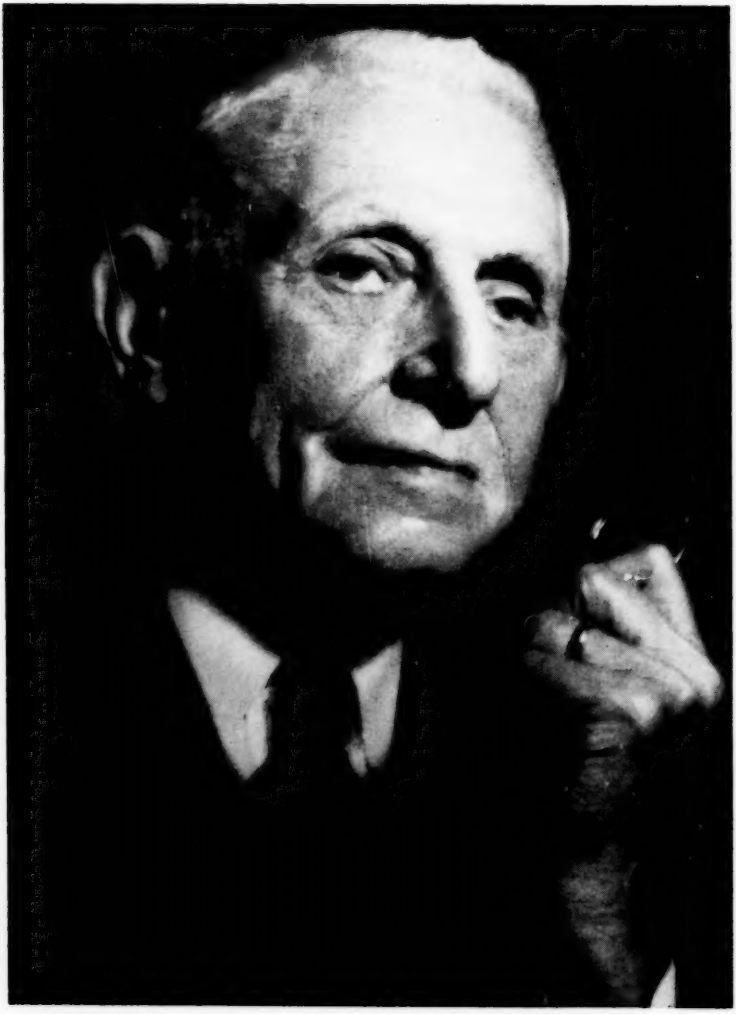

Torald Sollmann

WHY AN ANNUAL REVIEW OF PHARMACOLOGY?

By Torald Sollmann

Western Reserve University, Cleveland, Ohio

My sincere thanks to the Editorial Committee for the opportunity to write a Bon Voyage! to the new *Review* as it sets out on its journey of adventure. "Annual," of course, only in the sense that it will be published every year, not that it will confine itself to what has been published in the current year. I trust that it will have a much broader base; that it will aim to review present tendencies in the light of their development.

And why do we need such a review of pharmacology? Let us start with fundamentals: What is "Pharmacology"? There are those who consider it the use of drugs, against disease and in poisoning. I prefer a broader definition: The reactions of living substance to chemical changes in the environment. That is surely broad enough. It covers large sections of biology, microbiology, physiology, biochemistry, biophysics, and probably all the "bios" that the future can and will devise; even anatomy in its modern conceptions, if you please. Why not say "all science," while we are about it? All can be viewed as ancillary to pharmacology—and vice versa. Even those who, for simplicity, would prefer the pragmatic concept of "Drugs and Poisons," cannot go very far without invading and infringing on these various disciplines. The boundaries between them are artificial and arbitrary, like the boundaries between the nations of a continent. But these have different customs, different governments—all arbitrary and subject to change. Ah, but the nations have different languages, which are not intelligible to the others without interpreters or special training, which is one reason why we need reviews. Another reason for such a broad concept is that it takes away the feeling of poaching, when the work of the investigator tagged with one of the arbitrary disciplines "transgresses" into the domain of another "department." In short, the pharmacologist is interested in all these fields, needs them, and, therefore, works with and in them. All the better.

But there are difficulties in this extension: difficulties of multiplicity, and of technicality. As to multiplicity, pharmacology, even in the restricted sense, has grown with the years in geometric progression, far exceeding the fertility of our best guinea pigs and rabbits, and so have the cognate sciences. Long ago Faraday wrote that if he tried to read all that was published in his field, he would have no time for other research, and it is this other research that makes progress. We are faced with the same problem, only vastly more gigantic.

Then we are faced with another specter, Technique. No longer do we work with cork, sealing wax and string, smoked drum, mercury manometer and Marey tambours, and the simple compound microscope. Modern concepts, modern apparatus require special engineering training beyond the

understanding of the profane. And language yet! For the nonspecialist much of that, often the most essential, is esoteric. He does not know the grammar, the words, even the alphabet. Most of the technicality is a necessary price of growth. We may rejoice in this growth—indeed we may, and we may groan—and indeed we do, but we have to do something more about it than rejoice and groan. To that there is only one answer: Divide and conquer! Leave it to one specialist to dig into one subject, another into another. Let them serve as interpreters in the first place, and then as guides who may show us glimpses of the landscape, and then maybe as leaders who give us an outline map of the territory and point to where we may go from there. With such an outline map before us, we may choose our path intelligently and push forward with some confidence. They may also guide us from uselessly exploring territory that has been virtually exhausted; although we may be sure that even the best worked mine may still contain nuggets of gold that previous explorers, with their eyes on their special objectives, have overlooked. Following even a well-beaten path, you are fairly certain to come upon something new—if you have the nose for it, but there is also a fatal chance of getting bogged in trivialities.

This holds especially for pharmacology. There are incalculably many chemicals that may change the environment of living substance, and in so many cases their study adds nothing substantially new. So many are practically the same thing, but they hold the temptation of playing them up as something new, something better; at least the same thing; or, anyhow, no worse.

Half a century ago, some of us had the idea that there already were too many drugs to be studied thoroughly and used well, and that it would be a gain if the less useful were dropped and attention concentrated on the more useful. The fundamental idea was right, but it should not militate against the everlasting search for new drugs that are valuable. And what valuable new drugs have been found that were beyond the dreams of those days! Some have been discovered by a keen scent, by luck, by trying many things and holding on to the best; the second-best will eventually sink. As an old philosopher remarked: If you wish to develop a good flute player, you must have a nation of flute players, most of them not so good. If only we adhere strictly to the facts, to truth, things will find their own level.

But in the meanwhile, all the more need for these specialists whose reviews may guide us through the labyrinth to the facts, to the truth. Since it is not possible for any of us to read all that is being published on every subject, we must make a practical compromise. Let us be thankful that there are guides who will interpret for us the topics in which they feel competent.

Here I am impelled to indulge in a personal nostalgic note: I was elected to the Physiological Society in 1902, and had the privilege of helping in founding the Biochemical and the Pharmacological Societies. The three usually met at the same place and time, with mutual contacts, and no conflicts of space—we were only a handful that any lecture room could

hold with room to spare. And incredible as it may seem, everyone could understand what everyone was saying, and talk about it freely. The meetings were really friendly affairs, almost a *Kaffee Klatsch.* You came away refreshed. Now there are sections innumerable, and you go away exhausted.

But that is the way it had to be; indeed, it was the way we all wanted and still want it to be. And when we do not understand, we trust that the *Annual Review* will tell us what is said and written. Let them come, and we shall follow them the best we can. More power to them! And so, may this *Review* strive to be what we all need, and thrive accordingly.

Postscript

I thought I had finished, but Dr. Cutting insisted that I must add some autobiographical reminiscences of those early nostalgic days when I entered on the scene of pharmacology. The readers may pardon the personal note. It was his idea, not mine. So here goes!

I was born, as is self-evident; I still live, as is equally evident. I worked, *tant bien que mal,* or I tried to. *Voilà tout l'histoire.* But Dr. Cutting insists on detail.

My birth was on February 10, 1874. It was in Coburg, one of those picturesque towns, like Weimar, which is located in a small ducal German state, with the renaissance architecture of Rothenburg, a medieval castle on a hill (like the Wartburg), parks, fields and forests, and a shallow little river—the Itz(istula). My father had been a schoolteacher—as some eight generations of his forbears before him. Now he held the office of *Stadtkirchner*—registrar of the protestant diocese and curator of the great medieval church, the Moritzkirche, in whose shadow—or light—we lived. The office took only part of his time, but he was naturally a worker and a naturalist with a scope ranging from microscopy to fossils. He published a monograph on edible mushrooms, another on the tongue of snails, on the sting of bees, on the sex life of trichina. He took a daily walk into the woods and fields; and by his side, or before or after, trotted his little son, as soon as he had learned to trot, and absorbed some of his lore, his love of nature, his love of knowledge; and before the boy had reached his tenth year, he had decided on his career; he would be a *Naturforscher,* a searcher into Nature. This was confirmed by his dislike for the formal grammar studies when he progressed to the gymnasium. It was a good gymnasium, dating back to the Reformation, and had the honor of graduating Goethe's father. I liked it, liked my schoolmates, liked my teachers, and got along well enough; but I preferred the great outdoors; and besides, I had other plans. My two older brothers had emigrated to "Amerika," that glorious land of opportunities, and gone into business. The oldest had married and owned a drug and bookstore in a small Ohio town, and he had plans for me. I must become a doctor! When I had the grounding of three or four years of Gymnasium, I must join him as an apprentice in America, and he would see to the rest of my education himself. Here was a prospect, a dream, sure to entrance a boy of 13 with an adventurous mind. America—maybe

Indians!—and study of facts instead of declensions and conjugations. Not a moment's doubt as to my decision. My parents consented, and so at the age of 13½ I formally cancelled my obligations to the Kaiser, and vice versa, and took ship with my brother, who was traveling in Europe, and High-ho on the bouncing main—and bounce it did indeed.

The drugstore experience was good for me, and in a few years I passed the Stateboard in pharmacy. Meanwhile my brother directed my studies, including botany, for the real goal was still before us. My brother decided that the next step toward this should be analytical chemistry, and with my informal educational background, he decided on Paris. To Paris I went and had the good luck to be accepted as special student at the chemical laboratory of the Military Hospital of Val de Grace, and at Paris I completed my premedical education—and learned more besides.

A small-town youth of 19 on his own in Paris!—in any cosmopolitan center for that matter, but Paris especially. The contrast between the restricted opportunities of a small, American town and the innumerable opportunities of the metropolis of France: the galleries and museums and open lectures; the new intellectual world in the making; the fellow students and other friends, from all over the world; the intimate talks in the cafés; the long walks through the boulevards, through humble streets packed with old apartment houses packed with people, living their complex lives; walks through trim parks with neat children and uniformed nurses; walks along the *quais* of the Seine, browsing through the bookstalls. It was enchantment, the Arabian Tales of the West.

That lasted a year and a half; then a tour through much of Europe, much of it on foot, knapsack on back, like a wandering scholar of old. Then, the boat to New York, the train to Ohio, and the medical school—where I entered in the fall of '94, and spent the next 50 years, the rest of my working days. This was indeed a lucky stroke, if not a special providence. In those days, medical teaching was generally an avocation of clinical practitioners. Few medical schools had funds to "hire" full-time trained teachers, but Western Reserve School of Medicine had recently received a liberal endowment to make the mare step more lively and was just engaging young men in the medical sciences to teach and do research. As the year opened, there arrived George N. Stewart in physiology (including histology and physiological chemistry, according to the English system), trained at Edinburgh under Rutherford, at Berlin under Du Bois-Reymond, at Strassburg, Manchester, Harvard. William T. Howard in pathology and bacteriology, trained at Hopkins under William Welch. Charles F. Hoover in clinical diagnosis, trained in Munich, Berlin, and Vienna. Carl A. Hamann, in anatomy, had arrived the year before from the University of Pennsylvania. Their common interest in scientific medicine drew them together in a congenial quartet. I was fascinated, attended their classes, besieged their laboratories. They soon adopted me into their circle, allowed me to share their interests and to join in their teaching; the Elysian fields spread out resplendant before me. Clinical classes may have been somewhat

neglected, but I managed to "pass"; work was a pleasure; days were long and nights were short; and the next year I was regularly launched as "demonstrator of physiology and histology" under Stewart's guidance. My fate was decided. I graduated and continued with Stewart, rising grade by grade, trusted with more and more responsibility.

Meanwhile Pharmacology was experiencing birth pains emerging from the womb of didactic "Materia Medica and Therapeutics." John G. Spencer, who studied under Schmiedeberg, was given funds to equip a teaching laboratory in an unused attic, but left for another school. After some rather abortive attempts, the faculty assigned it to me in the spring of '98, and I started a regular lab class, improvising the day's work as I went along. This succeeded so well that the faculty gave me a title in Pharmacology, relieved me of my duties in Physiology, and generously supplied me with funds to spend a semester or two at the Strassburg Pharmakologische Institut—the seedbed of pharmacologists—to find out what it was all about, to see how German institutes were run; above all, to meet some of the men who were making medical science and to see what made them move and tick. I carried a "project" or two with me as a back-log, picked up others that would give me some insight. When the University closed for summer vacation, I shouldered my knapsack, roamed through Germany and the Alps, took ship—and felt myself launched as a *Fachmann,* a pharmacologist by trade and profession.

And so, after many circuitous irrelevancies, we have at last arrived at the starting point of this Review—the Status of Pharmacology at the Turn of the Century. Actually, so far as I know, nothing relevant to pharmacology occurred at midnight of 1899, except the degradation of an uncertain amount of C_2H_5OH to CO_2, and of this only a relatively small, but not negligible, proportion by pharmacologists. Modern scientific pharmacology took its birth as an offshoot of experimental physiology a century earlier with Claude Bernard and was formally recognized, about a half a century later, by the foundation of an *Institut* at Dorpat under Buchheim, followed by the Schmiedeberg Institut at Strassburg. These are notable landmarks in a vista that stretches back beyond antiquity to Paracelsus, the Arabs, the Greeks; to the wise women or witches; to the magicians, wizards, and shamans. Indeed its driving spirit, curiosity, extends even to lowest animals—perhaps to the plants, the atoms, the electrons, the *Urstoff* itself. But where does that get us? To the Infinite, the Endless. A paper in the Review must have an end as well as an aim. Where were we when we got lost? Oh, at the turn of the century—only that, a mere sixty years ago when Pharmacology was struggling for official recognition in this country, and people asked (as some do still), "Pharmacology?—is that a new name for Pharmacy?" In Europe, thanks largely to Schmiedeberg and his Institut, and the Naunyn-Schmiedeberg Archives, this question of status had been officially and practically settled ("implemented," we would say now). In this country the subject had struck root through Abel and Cushny and their pupils, and (modestly) here at Western Reserve, and was sending

vigorous cuttings (no pun intended, ***honi soit***) through the land. All searching, and teaching, and searching, ever-searching: What do drugs do, and how do they do it? First, the ***what***, then the ***why***, and the ***how***, and many a rub, rub-a-dub-dub between. There were plenty of drugs in the pharmacopeia; plenty more in the drug shops; plenty had been written about them, was being written about them. But what did they do really? Clinical experience was always an incomplete and sometimes a false guide—it could not be controlled; the conditions could not be varied—until it was verified by the advancing methods of experimental physiology—respiration, circulation and intestines on rabbits, cats, and dogs; muscle, heart, capillary flow on frogs; lethal dose and chronic effects on guinea pigs, rats, and mice. No end of good work to be done, and at Woods Hole, the wealth of lower organisms from starfish ova to limulus. Each acquisition a steppingstone to the next; each problem answered posing other problems. Concepts and experimental techniques advancing day by day. Such was the exciting prospect, such the plodding practice, then as it is today.

New drugs, new active agents, came along; hormones, vitamins, synthetics. The ***what*** brought its rich harvest. The ***how*** too became clearer. Knowledge extended into depth as well as breadth. Abel, particularly, insisted that pharmacology rest on a chemical basis. As drug-apprentice, I had often wondered why strychnine should cause convulsions when quinine did not, why arsenic should be so much more fatal than salt. The discovery of enzymes, those leprachauns of the chemical world, later revealed glimpses into the ***how***, if not yet into the ***why***. Biological exploration of the endocrines brought the isolation of the hormones, the concept of their regulation of body functions, down to the transmission of the nerve impulse. Step by step we are still climbing the ladder, to where more and more of the ***how*** and of the ***why*** can be seen. In nutrition, the vitamins entered the field, each with its lantern.

Always, with better understanding of drugs—incomplete as it was—came new and better drugs. Organic synthesis was still an infant at the turn of the century, relatively small, but lustily growing, always growing. Ever more and more chemicals in need of pharmacological investigation. It is tempting to follow such topics as the evolution of antisyphilitic treatment, from mercury to arsphenamine to bismuth to penicillin, of suprarenal extract through to the latest corticoids, and so many more, but such may be the function of special articles in the *Annual Review*.

As we finally try to sum it up, the intellectual world of Pharmacology, at the turn of the century, was fundamentally what it is now, although so much has changed. We can read the great Book of Nature better with fluorescent lighting, than with the old kerosene lamps: chiefly and fundamentally, Light is Light; we must strive for *Mehr Licht*, and to this the *Annual Review of Pharmacology* is dedicated.

HIGHLIGHTS OF PHARMACOLOGY IN JAPAN[1,2]

By Hiroshi Kumagai
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AND
Hajime Yamada
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Since 1952, studies on the factors regulating ATP contraction of muscle models, especially of glycerinated muscle fibers, have been energetically conducted at the Department of Pharmacology, University of Tokyo for the primary purpose of inquiring into the mechanism of so-called "excitation-contraction coupling." In 1955, Kumagai, Ebashi & Takeda (1), starting with the crude relaxing factor of Fujita (2), were able to identify the essential relaxing factor as the sediment fraction obtained by centrifuging at 18,000 g for one hour. Emphasis (3, 4, 5) has been laid on the identity of the fraction with the preparation of Kielley-Meyerhof's ATPase. Further development of this work was recently done by Ebashi & Lipmann (6) at the Rockefeller Institute. According to their findings, the fraction of essential relaxing factor identified as a vesicular component (that is, the sarcoplasmic reticulum) can combine calcium in an ATP-linked reaction stronger than ethylenediaminetetraacetic acid. This calcium binding is considered to represent a mechanism for accumulating inorganic ions in living organisms. On the other hand, Ebashi (7) has revealed the essential role of a minute amount of calcium in the mechanism of actomyosin syneresis with ATP, which is in good accord with the results of Weber (8) derived from the studies on actomyosin ATPase.

On the basis of these findings, Ebashi has postulated that the relaxing action of the sarcoplasmic reticulum is due to its extraordinarily strong calcium-binding activity, i.e., calcium located in the vesicular structure is the key substance for the excitation-contraction coupling.

Since 1940 a series of researches have been carried out in Japan on the toxicity of the yellowed rice polluted by *Penicillium toxicarium* [Miyake, Naito & Tsunoda (9)]. In 1948 yellowed grains were also found in rice imported from Spain, as a result of pollution by *Penicillium islandicum* Sopp. Kobayashi, together with Uraguchi *et al.* at the Department of Pharmacology, University of Tokyo, began to investigate the toxicity of these polluted rice grains and to identify the toxins involved (10 to 13). Quite recently Kobayashi, Uraguchi, and associates (13 to 16) have successfully

[1] The survey of the literature pertaining to this review was concluded in April, 1960.

[2] Abbreviations used in this chapter include: ATPase (Adenosine triphosphatase).

isolated two kinds of active principles from the toxins produced by *P. islandicum* Sopp. One is a chlorine-containing peptide, and the other a yellow pigment named luteoskyrin; both were hitherto unknown. Repeated administration to mice and rats has shown that these toxins may produce cirrhosis of the liver. In addition to these findings, Kobayashi, Uraguchi *et al.*, in collaboration with Miyake, Saito, and their collaborators at the Department of Pathology of the University of Tokyo, have also demonstrated in rats by long-term feeding (up to 622 days) that the yellowed rice inoculated by *P. islandicum* Sopp is capable of inducing primary hepatic carcinoma. These findings may throw light on the problem of high incidence of hepatic carcinoma in Orientals.

In 1938 Okada & Mimura (17) devised a method of vital staining of hard tissues. In this method a small quantity of lead acetate is injected into an animal, and the lead deposited in hard tissues is histochemically shown as a distinct fine line. By this means, it became possible to mark the lapse of time in hard tissues, such as teeth or bone, in a living body under desired experimental conditions. Numerous results have already been published (18). For vital staining, a solution of lead acetate is injected into the ear vein of a rabbit; the animal is sacrificed after an appropriate lapse of time, and its teeth are removed and fixed in formaldehyde solution. The teeth are decalcified in 0.2 *N* hydrochloric acid (acetic or phosphoric acid may be used), saturated with hydrogen sulfide, and frozen sections are prepared in order to detect lead lines on dentine. In the decalcified section of the dentine, there is a striated pattern around the pulp cavity, like annual rings in wood, that is stained by hematoxylin. The layers stained by this dye are formed during the night, and those not stained are formed during the day. It was shown that incisor dentine of the rabbit is being formed incessantly at the rate of about 25 μ per 24 hours and calcium metabolism *in vivo* is thus recorded as a wave pattern in dentine. Okada & Asoda have also discussed the authenticity of hematoxylin staining of decalcificated dentine, which is taken as the indication of the degree of calcium deposition in the dentine method. They (19) have shown that the calcium deposition in dentine is the function of blood level of calcium (especially of calcium ion) and of inorganic phosphorous, and that dentine is a far more sensitive indicator of calcium deposition than bone.

Nakao *et al.* at the Department of Pharmacology, Tokyo Jikei-Kai Medical College have been engaging in the studies of biotransformation of steroids in living bodies and tissues. [In this review only one (20) of their recent contributions will be cited.] Their studies are concerned with the identification of the steroids in the human sweat secreted under high environmental temperatures. The experiments were conducted on healthy human subjects of 20 to 30 years of age in a perspiration chamber, and the sweat was collected by the method devised by Takamatsu (21). The room temperature was kept at 38° to 40°C. for four hours and 42° to 45°C. for two hours. The humidity was kept at 70 to 90 per cent. They obtained four

steroid fractions by paper chromatography. Two of them were identified, whereas the quantities of the remaining compounds were too small for complete identification. The toluene-propylene glycol system of Zaffaroni, benzene-methanol-water system of Bush, and ligroine-*tert.* butanol system of Eberlin and Bongiovanni were used for development. One compound was proved to be Δ^4-pregene-11β, 17α-, 20-triol-3-one. This compound was shown to have an antidiuretic action in water-loaded rats. The second steroid was identified as a compound having a Δ^4-3-keto group and an α-ketol group at C17. Although they have not yet been able to confirm the presence of aldosterone in human sweat, they presume that this second compound might be a metabolite closely related to aldosterone.

In 1938 at Osaka University's Department of Pharmacology, Imaizumi (22) expressed the view that some oxidation and methylation process on the side chain of DOPA might occur in the adrenal gland, because he had demonstrated the presence of an epinephrine-like pressor substance in adrenal tissue after the decarboxylation of DOPA in the liver. After further studies, Imaizumi now postulates that besides the main pathway of epinephrine biosynthesis from DOPA by way of hydroxytryptamine and norepinephrine there is another possible pathway (written as tyramine → hydroxytyramine → norepinephrine → epinephrine) because his co-worker Koyabu, recently found a copper-containing enzyme, which is capable of oxidizing tyramine to hydroxytyramine, in rabbit serum and liver.

It is of interest to note here Imaizumi's turn-over theory (23) concerning the mechanism of epinephrine sensitization by amino acid in the blood vessels. According to Imaizumi's theory, epinephrine is first oxidized to epinephrinequinone which in turn acts as an acceptor of hydrogen derived from the oxidation of amino acids. In this way amino acids accelerate the turn-over rate of epinephrine ⇌ epinephrinequinone. He also postulates the same mechanism for ascorbic acid which acts quite similarly to amino acids in this respect.

The brilliant work done by Okamoto at the Department of Pharmacology and Research Institute of Tuberculosis, Kanazawa University should not be overlooked. In 1939 it was found by Okamoto (24) that hemolysin (streptolysin S) production of hemolytic streptococci is markedly enhanced by the addition of yeast nucleic acid to the culture medium. This striking effect, designated as "RNA-effect," was confirmed and extended by many other research workers [Bernheimer (25); Egami (26); and others]. Among findings connected with the RNA-effect, those to be mentioned here are: (*a*) The RNA-effect appears to be specific for beta-hemolytic streptococci and is not shared by any other bacterial species so far examined, with the exception that tobacco mosaic virus ribonucleic acid, ribonucleic acid from certain other sources such as mammalian liver, wheat, and bacteria, and Ochoa's biosynthetic poly-ribonucleotide of adenine, guanine, uracil, cytosine (1:1:1:1) type (27) were shown to be effective in streptolysin S formation. It was thus supposed that hemolytic streptococci and ribonucleic

acid are in close metabolic relationship with each other. (*b*) 2,2′-Dihydroxy-azo-benzene and its tetrabromo derivative were found to be highly inhibitory against the formation of streptolysin S—a finding which suggests that the compounds might also act as potent inhibitors of nucleic acid metabolism in other living cells. (*c*) If well-washed living hemolytic streptococci are made to act *in vitro* on Ehrlich ascites carcinoma or Yoshida ascites sarcoma cells or even leucocytes, an appreciable amount of streptolysin S is produced. In this case, the toxin may be regarded as being produced at the expense of ribonucleic acid contained in the tumor cells or leucocytes.

With these facts in mind, Okomata *et al.* are now conducting two kinds of anticancer studies (28). The first type of experiments are with hemolytic streptococci. Evidence has been provided by Koshimura *et al.* to show that living hemolytic streptococci, regardless of their virulence for mice, have a characteristic ability to affect tumor cells *in vitro* and *in vivo*. Since streptolysin S alone was found to be entirely ineffective in this respect, it was inferred that the anticancer effect shown by the cocci is mainly attributable to their ability to affect the ribonucleic acid in tumor cells. Recently, these workers have successfully prepared cell-free extracts of the cocci, which may exhibit either RNA-effect (streptolysin S formation) or anticancer activity. It is of interest to recall here earlier reports concerning the observations that complete or partial regression of malignant tumors was noticed during and after intercurrent streptococcal infections (Busch, 1886; Fehleisen, 1882-83; Bruns, 1888; Coley, 1891; etc.). The second group of anticancer studies are those experiments on the carcinostatic activity of 2,2′-dihydroxy-azo-benzene derivatives. A number of compounds in the 2,2′-dihydroxyazo-benzene series were synthesized by Hirata; among them, Azo-106 bis(2-hydroxy-3,5-dibromophenylazo)-n-propylphloroglucinol, a potent inhibitor of RNA-effect, was shown to be especially effective against Ehrlich ascites carcinoma, Sarcoma 180, and Yoshida ascites sarcoma.

The working team of Kyoto University has made a distinct contribution to the neuropharmacological approach to the study of the site of action of analgesics. The action of morphine and its congeners on the various afferent pathways of pain was studied by Fujita *et al.* (29, 30) using electrophysiological techniques. In the cat, these investigators showed that morphine (6 mg./kg.) and meperidine (12 mg./kg.) blocked splanchnic afferents in the spinothalamic tract and vagal afferents in the medulla oblongata, but did not block afferents from the sciatic nerve. The same doses of morphine and meperidine abolished the second component of cortical potentials evoked by local cortical stimulation, recruiting responses evoked by stimulation of thalamic intralaminar nuclei and augmenting responses evoked by repetitive stimulation of the medical lemniscus. Yamamoto (31) reported that the site of action of Ohton, 3-dimethylamino-1, 1-di-(2′-thienyl)-1-butene, a potent morphine-like analgesic, was the same as that of morphine. Matsumura *et al.* (32) observed the synergism between morphine and methamphetamine in depressing the cortical potentials evoked by splanchnic stimulation, as well

as recruiting responses and augmenting responses in the cat. In the cat and rabbit, Fujita *et al.* (29) and Yasuhara (33) reported that chloral hydrate (100 mg./kg.), urethane (400 mg./kg.), and the inhalation of ether abolished cortical potentials evoked by sciatic, splanchnic, and vagal stimulation, and that barbiturates did not abolish these potentials. Takagi *et al.* (34) and Ogiu *et al.* (35) have studied the action of morphine and meperidine on the reflex arcs of spinal cord using the intact cat, thalamic, midbrain, high-spinal, and low-spinal cat. In all of the preparations except the low-spinal cat, these investigators found that morphine (7 mg./kg.) depressed both the monosynaptic and multisynaptic reflex discharges evoked by single shocks applied to the ipsilateral sciatic nerve. In the low-spinal cat (transected at T_1–T_2 or L_2–L_3 levels) no inhibitory effect of morphine was observed, even after 14 mg./kg. of morphine. In addition, those investigators found that in cats with lesions in the brain-stem reticular inhibitory area, morphine enhanced both types of reflexes; whereas in cats with lesions in the reticular facilitatory area, morphine abolished the polysynaptic reflex discharges and reduced the monosynaptic reflex discharges. These findings suggest that morphine affects spinal reflex indirectly through stimulation of facilitatory and inhibitory centers in the brainstem, with inhibitory effects usually predominating. Similar results were obtained with meperidine.

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HIGHLIGHTS OF PHARMACOLOGY IN LATIN AMERICA[1]

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Many factors have retarded the development of modern pharmacological research in Latin America. Of these, the most important have been the scarcity of full-time research positions in academic institutions; the risks in undertaking long-term projects under conditions of social and political unrest; the almost total lack of private and direct official grant support for research; and the lack of a strong, autochthonous pharmaceutical industry which, elsewhere, is the source of new drugs for pharmacological research and of stimulus for the training of pharmacologists. Notwithstanding these factors, or perhaps because some of them are ceasing to be operant, there has been a substantial increase in the volume of original publications in the field during the last few years. This is best illustrated by the following yearly breakdown of the distribution of the 490 papers which constituted the preliminary selection for this review: 1952, 22 articles; 1953, 27; 1954, 28; 1955, 36; 1956, 48; 1957, 69; 1958, 82; 1959, 142; 1960, 36 (the figure for 1960 represents publications through February of that year). The first selection was made with no bias in favor of publications from the later part of the period. It is as yet a very modest contribution from a large geographical area with a traditional interest in the action of drugs of the type represented by the sixteenth century, monumental contribution of Francisco Hernández, whose complete works are currently being edited by the Universidad Nacional de México (30).

Certain fields of active pharmacological research will not be discussed, inasmuch as recent reviews by the investigators principally involved are available. In 1956, Braun-Menéndez (6) reviewed the pharmacology of renin and hypertensin. It is of interest that the two groups of workers most actively engaged in this field have agreed to use the term "angiotensin," instead of hypertensin or angiotonin, to refer to the vasoactive polypeptide which results from the action of renin [Braun-Menéndez & Page (7)]. Rocha e Silva (60) has recently reviewed his and his collaborator's contributions to the pharmacology of bradykinin. Finally, reference should be made to three reviews by Caldeyro-Barcia and co-workers (9, 10, 11) which summarize the contribution of the Uruguayan workers to the study of the actions of oxytocin and other substances on the pregnant human uterus.

Interest in problems related to theoretical and quantitative pharmacology

[1] The survey of literature pertaining to this review was concluded in February, 1960.

has been apparent. Croxatto & Huidobro (19) have made a general presentation of their theory regarding the dependence of pressor or depressor effects of sympathomimetic amines on the "surface complementarity" between the drugs and two hypothetical types of receptor sites for which they offer shadow models of configuration. Rocha e Silva & Rothschild (63) have proposed an ingenious and relatively simple procedure for assaying materials which induce tachyphylaxis. The decrease in response attributable to repeated administration is confounded with the order of administration in a standard Latin square design using four similar test objects. More recently, Rocha e Silva (61) proposed an indirect method for calculating pAx based on the ratios between the slopes of reciprocal dose-effect regressions obtained in presence and absence of the antagonist. The same investigator (59), referring to the kinetics of recovery from inhibition by antihistaminics, atropine, and antispasmodics, suggests that the process of recovery is of an autocatalytic nature and not to be explained on the basis of the type of breakdown of the drug-receptor complex which can be predicted from mass-action considerations.

Pardo & Magaña (51) and Pardo (49) have suggested the possibility that, of the phenomena loosely grouped under the heading of tachyphylaxis, the classical type seen, for example, after the repeated administration of sympathomimetic amines may be thought of as depending on the lack of correspondence between the fraction of receptors occupied by a drug and the fraction of receptors participating in the process of activation. This disparity would arise from the brevity of the disturbance created by receptor occupation in comparison with the relatively long life of the drug-receptor combination. Autoinhibition of this type would be expected even in the case of the normal mediators. In relation to this possibility, Pardo, Magaña & Vargas (53) have presented evidence that tachyphylaxis of the type mentioned above may constitute a normal mechanism for the regulation of responses to catecholamines. In order to handle quantitative data from systems in which autoinhibition is important, Pardo & Magaña (50) have analyzed the general problem of dose-response relations and have proposed a general formula for use both in the case of responses of an all-or-none type and for responses of variable magnitude. The proposal implies that all biological responses can be thought of as being of a quantal nature, that the sensitivity of effector units referred to the number of receptors needed to be activated for response is distributed normally, and that the classical hyperbolic relation between the concentration of the drug and the fraction of receptors occupied is tenable. The same investigators (52) have offered experimental data in partial adequation of their hypothesis. Indirect evidence for the distributive nature of effector sensitivity has also been derived from the study of the pattern of action of several sympathomimetic amines on the vascular responses to catecholamines [Magaña & Pardo (35)].

Referring to the pharmacology of catechol amines and of drugs that modify their effects, García Ramos (28) reported that epinephrine greatly

increased the oxygen consumption of isolated strips of intestine and suggested that the smooth muscle relaxation produced by this agent is related to the acceleration of lactic acid production. Rocha e Silva, Corrado & Ramos (62) found that reserpine and sympatholytic agents prolong the vasodilator action of bradykinin. Pardo, Magaña & Villarreal (54) have reported that the nictitating membrane of reserpinized cats, though more sensitive than that of nonreserpinized animals to small doses of injected epinephrine, is none the less much more susceptible to transmission fatigue when stimulated at moderate frequencies through its nerve supply. They suggest that this effect may constitute the basis for the antiadrenergic actions of reserpine on structures in which tonic activity is normally maintained through repetitive stimulation.

Of the cholinergic structures, attention has been given to the secretory glands of the gastrointestinal tract. Vidrio (66, 67) described a useful semicontinuous method for measuring gastric secretory activity and has applied it in order to re-evaluate the effects on secretion of a group of blocking agents and a variety of cholinergic agents. Junqueira, Rothschild & Vugman (34) have suggested that the main action of atropine on the pancreatic exocrine secretion elicited by parasympathetic stimulation is to block the extrusion of zymogen granules. They were unable to find any interference with the synthesis of zymogen granules or with the rate of amylase resynthesis.

Work has been done on the general question of possible selectivity of action of ganglionic blocking agents on sympathetic and parasympathetic structures. Cato, Vélez & Nájera (12) have shown that selectivity for parasympathetic ganglia of the type initially reported for ethyl-methyl-isooctenyl amine, a tertiary amine [Pardo *et al.* (55)], could also be demonstrated for several classical ganglionic blocking agents when their effectiveness in blocking the heart-rate responses to vagus stimulation and the pressor responses to carotid clamping was compared. The probable inadequacy of using effector response as a criterion for ganglionic action led Alonso-deFlorida *et al.* (3) to compare the actions of several blocking agents on the postganglionic action potentials at the superior cervical ganglion and at the ciliary ganglion. They found that the sensitivity of the two structures to the different substances was very similar, and that the only difference was a greater tendency for the ganglionic blocking agents to produce facilitation at the ciliary ganglion at very low dose levels. In a later communication, Alonso-deFlorida *et al.* (4) have reported that an analysis of the effect of tetraethylammonium on synaptic transmission indicates a greater subliminal fringe after maximal preganglionic stimulation in the case of the ciliary ganglion as compared to the superior cervical ganglion.

Additional information regarding the ganglionic effects of drugs is found in the report by Corrado (16) of ganglionic blockade by large doses of streptomycin, in the study of Prado & Carlini (58) on the influence of ganglionic blockade on the actions of hypertensin, and in the report by Magaña,

Ugalde & Pardo (36) of ganglionic blocking activity for several sympathomimetic amines. Mention should be made of the older communication by Middleton *et al.* (43) on stimulation by acetylcholine in the apparently ganglion-free atropinized heart papillary muscle preparation.

In the field of neuromuscular transmission, Chagas (14) has recently reviewed his and his collaborators' work which led to the isolation from electric organs and mammalian tissues of acid mucopolysaccharides which may be identified with the macromolecular substance supposedly responsible for curare fixation in the intact structures. Using radioactive curares and curare-like agents, Braun-Cantilo *et al.* (5) have observed that the organs retaining most of the injected drug are those which have the highest content of mucopolysaccharides. Also in this area, neuromuscular blocking properties have been reported for streptomycin by Vital-Brazil & Corrado (69) and for neomycin by Corrado, Ramos & deEscobar (17). Reference should also be made to the careful analysis of the action of scorpion venom on the neuromuscular system carried out by del Pozo and collaborators some years ago and recently reviewed by him (22).

Contributions dealing with different aspects of the pharmacology, release, and blockade of histamine have appeared recently. Naranjo & Banda de Naranjo (47) have reported that the pressor effect of histamine in the rabbit may not be related to epinephrine release, since adrenalectomy, adrenergic blockade, and ganglionic blocking agents do not modify this response. Mota (44) has shown that histamine depletion produced by the chronic administration of compound 48/80 protects animals from anaphylactic shock. Moussatché & Provoust-Danon, working with guinea-pig lung slices, have observed that succinate favors and malonate interferes with antigen-induced histamine release (45) and that neither substance significantly alters histamine release induced by compound 48/80 (46).

In a carefully instrumented study of the effects of K-strophanthoside and lanatoside C on atrial muscle, Méndez & Méndez (40) found that the glycosides, at therapeutic levels, reduced atrial excitability and slowed conduction velocity. A discussion of the significance of this and previous studies of the physiological actions of cardiac glycosides has been published [Méndez & Méndez (41)]. More recently, Aceves, Méndez & Méndez (1) and Méndez, Aceves & Méndez (38) have reported that digitoxin and acetyldigitoxin antagonize the increase in heart rate following epinephrine injection or stimulation of the adrenergic nerves. In a separate paper, Méndez, Aceves & Méndez (39) reported that the digitalis increase in the refractory period of the A-V node is mediated, in part, through peripheral inhibition of the effects of adrenergic tone. Recently, Méndez & Rodríguez (42) have focussed new interest on the antifibrillatory actions of spartein. Since then, Fuentes published papers on the antagonism between spartein and veratrine (26) and on the antiaccelerator effects of spartein (27). Esperanza (23) has successfully used the the intra-auricular injection of very high doses of

this alkaloid in the treatment of ventricular fibrillation in anesthetized patients undergoing cardiac surgery.

Among other contributions in the area of cardiovascular pharmacology, the following should be mentioned. Folle (25) has reported that the hypotensive effects of quinidine are principally attributable to the adrenergic blocking actions of the alkaloid. Cesarman (13) and Cossio (18) have published reports on the former's original observation regarding the possible usefulness of iproniazid in the treatment of angina pectoris. Villarreal, Magaña & Pardo (68) have reported that animals treated chronically with iproniazid are more susceptible to ephedrine tachyphylaxis than animals not so treated. They interpret their results as signifying occupation of adrenergic receptors by iproniazid. Comesaña *et al.* (15) and Nava *et al.* (48) have reported on the antihypercholesterolemic effects of nicotinic acid in dogs and in humans.

Croxatto, Rosas & Barnafi (20) have reported an increase in the renal excretion of water, sodium, and choride induced by purified oxytocin both in normal and hydrated rats. This response was abolished by hypophysectomy and restored by administration of sodium chloride and DCA [Croxatto & Zamorano (21)]. Cafruny & Vargas (8) have shown that a variety of vasodilator agents depressed mercurial diuresis. The same authors [Vargus & Cafruny (65)] have observed that *p*-chloromercuribenzoate, a nondiuretic organic mercurial, was capable of producing salt and water diuresis when kidney blood flow was kept at a constant rate. Pines has studied some cardiac actions of acetazolamide (56) and of chlorothiazide (57). He reports that the latter drug protects against arrhythmias induced by prednisolone and strophanthin.

In another area, Werner (70) and Timo-Iaria & Werner (64) have presented data which they interpret as signifying that some analeptic drugs facilitate impulse transmission in the reticular formation. Fernández & Roldán (24) have reported that atropine is capable of blocking the spread of convulsive activity elicited by cortical stimulation. Gutiérrez-Noriega & Zapata-Ortiz (29) have observed that cocaine produces marked inhibition of multisynaptic and moderate inhibition of monosynaptic reflexes, effects which are antagonized by short-acting barbiturates. Zapata-Ortíz, Castro & Campos (71) have reported that bemegride antagonizes the central nervous system action of barbiturates, urethane, and ethyl alcohol and that it may produce convulsions at low dose levels in animals treated with large doses of morphine. Matallana & Borison (37) have described a method for producing cough in unanesthetized decerebrate cats which has proved useful for the evaluation of central-acting antitussive agents. Alcocer, Eckhaus & Villaseñor (2) have found that epinephrine and norepinephrine prolong the slow negative potential resulting from physiological stimulation of the frog's olfactory epithelium.

Several contributions regarding the mechanism of action and the use of

hypoglycemic sulfonylureas have been published. Houssay & Penhos (31) and Houssay *et al.* (32) found that the presence of pancreatic tissue was necessary to obtain the hypoglycemic effect of these compounds and that adrenalectomized animals were very sensitive to this effect. In a separate paper, Houssay *et al.* (33) reported that sulfonylureas reinforced the hypoglycemic action of insulin both in depancreatized and in eviscerated animals and that, in the absence of insulin, no hypoglycemia could be elicited in these preparations. Zubirán, Domenge & Escobar (72) have studied the general human pharmacology of sulfonylureas and report evidence suggesting that these drugs act through the release or protection of endogenous insulin, or both.

Numerous contributions which refer to the clinical use of the sulfonylureas will not be reviewed, nor will mention be made of the rather abundant publications referring to the biological effects of other hormones. Papers reporting clinical investigations, on the chemotherapy of the infectious and parasitic diseases prevalent in the area have been considered to be outside the scope of the present review. Finally, research on potentially active products of natural origin has been sporadic, and no transcendental contribution can be commented upon.

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HIGHLIGHTS OF SOVIET PHARMACOLOGY[1]

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The progress of Soviet pharmacology has been closely associated with the development of a national pharmaceutical industry which may be said to have sprung up only after the Revolution, since such industry was practically nonexistant in tsarist times and since drugs, especially synthetic ones, were, as a rule, imported from abroad. The general industrialization of the Soviet Union brought about the creation of large chemical plants which, by the beginning of World War II, were able to supply the country with pharmaceutical products of Soviet make. To our pharmacologists fell the task of furthering the introduction of effective new drugs into industry and medical practice.

In order to meet the requirements of a newly created pharmacological industry there arose a number of scientific institutions. The first to be founded was the VNIKhFI (All-Union Scientific Research Chemo-Pharmaceutical Institute, Moscow) with Professor M. D. Mashkovski heading its department of pharmacology. A similar Ukrainian institute was created in Kharkov. The VILAR (All-Union Medicinal and Aromatic Plants Institute), situated in the vicinity of Moscow, has local branches in many districts of the U.S.S.R. Special institutes attached to the Academies of the Armenian S.S.R. (Erevan) and Latvian S.S.R. (Riga) are engaged in drug synthesis. After World War II the oldest medical research institute in the country, the Institute of Experimental Medicine, created a department of pharmacology, which is directed by the present writer. A few years ago the Academy of Medical Sciences in Moscow founded the Institute of Pharmacology and Chemotherapy, headed by Professor V. V. Zakusoff.

In addition, pharmacological research is carried on throughout the Union by the chairs of pharmacology of various medical and chemopharmaceutical faculties, or institutes. In tsarist Russia there were only 11 chairs of pharmacology; their total number has now risen to almost 80. The number of scientists working in this field has undergone a similar increase. Up to recent times Soviet pharmacologists formed a special section in the All-Union Physiological Society. The last conference of pharmacologists decided to create a separate Pharmacological Society attached to the Academy of Medical Sciences.

The most important review publishing original papers by Soviet pharmacologists is *Farmakologiya i Toksikologiya*, an English translation of

[1] The survey of the literature pertaining to this review was concluded in June, 1960.

which appears in the U.S.A. Moreover, there is a pharmacological section in *Byulleten' Eksperimental'noy Biologii i Meditsiny* issued by the Academy of Medical Sciences.

As in all other branches of science, pharmacological investigation in the U.S.S.R. is carried out on planned lines. The planning of pharmacological work, i.e., the selection of the problems considered most urgent, is accomplished by a special Problem Commission formed of the leading pharmacologists of the country and attached to the Institute of Pharmacology and Chemotherapy of the Academy of Medical Sciences. Such a plan embraces the most pressing problems of public health, such as search for drugs effective in the therapy of tumors, pharmacology of the cardiovascular system, investigation of neurotropic drugs, chemotherapy of infectious diseases, etc. The plan agreed upon by the commission does not, however, preclude private initiative on the part of individual laboratories—all scientific achievements are gratefully acknowledged and encouraged.

Space limitations prevent coverage of the whole field of Soviet pharmacology in a single short review. Accordingly, the present paper will be limited to problems more closely related to the work of this writer (namely, researches in the pharmacology of the nervous and cardiovascular systems) and, as a rule, will mention recent publications only.

The special interest shown by Soviet pharmacologists in the pharmacology of the nervous and, particularly, the central nervous system is, to a great extent, attributable to the influence of I. P. Pavlov's ideas. Energy has been focussed on the search for an investigation of agents blocking the transmission of nervous impulses in different portions of the reflex arc, since central and peripheral rest is of prime importance in effecting the cure of various pathological conditions.

Among the work in the pharmacology of the central nervous system, mention should be made of the investigations in the central cholinergic synapses, which were reviewed at the last International Physiological Congress in Buenos Aires [Anichkov (6)]. These investigations resulted in the isolation from the vast body of cholinolytic agents of a special group of so-called central cholinolytics, i.e., substances exerting a predominant blocking action upon the central cholinergic synapses. Besides well-known agents belonging to this category, such as adiphenine (Soviet equivalents: spasmolytin, diphacil), caramiphen (Soviet equiv.: pentaphene), benactyzine (Soviet equiv.: diazil), the following central cholinolytic agents were recommended in the U.S.S.R. for clinical use: tropacin (tropin ester of diphenylacetic acid hydrochloride); aprophen (β-diethylaminoethyl ester of diphenylpropionic acid hydrochloride); tiphene (β-diethylaminoethyl ester of thiodiphenylacetic acid hydrochloride); gangleron, synthetized by Mnjoyan (23) (α,β-dimethyl-γ-diethylaminopropyl ester of *p*-iso-butoxybenzoic acid hydrochloride); methyldiphacil (β-diethylamino-iso-propyl ester of diphenylacetic acid hydrochloride); methyldiazil (β-diethylamino-iso-propyl ester of diphenylglycollic acid hydrochloride). The central action of the sub-

stances pertaining to this group was proved by observations on patients, experiments upon the conditioned reflexes of animals, and electroencephalographically; their cholinolytic character was shown by their antagonizing the central action of anticholinesterases and cholinomimetics [Mikhelson *et al.* (20); Denissenko (11)]. It was furthermore observed that in accordance with the peculiarities of their peripheral action, some central cholinolytics, e.g., adiphenine and methyldiphacil, show preponderant antagonism to the central action of nicotine, whereas benactyzine and methyldiazil antagonize mainly the central effect of arecoline. The latter group exerts a selective blocking action upon the synapses of the efferent system of the reticular formation. The cholinoreceptors of the central synapses, as well as the peripheral ones, thus belong to two distinct classes, namely, nicotine-sensitive systems (or N-cholinoreactive, as they should be called), and muscarine-sensitive ones (M-cholinoreactive, according to our terminology). Central cholinolytics enhance the effect of hypnotics and narcotics, a fact used to great advantage in clinical practice [Anichkov & Denissenko (5)]. Their action upon reflex-induced trophic disorders is very important. Benactyzine, and, to a lesser degree, caramiphen, prevent, in animals, the formation of reflex-induced gastric ulcers that are caused by intense irritation of the duodenum. This effect does not depend upon the peripheral atropinomimetic action of the drug, since quaternization of central cholinolytics enhances their peripheral atropinomimetic properties and diminishes their action upon reflex-induced ulcers [Zavodskaya (33, 34)]. The regulating effect on trophic processes obviously depends upon the central action of the cholinolytic. One may suppose that this central action is part of their curative effect not only in the case of gastric, but in other internal diseases as well. Some central cholinolytics, such as adiphenine, tiphene, etc., stimulate hormonal activity in the adrenal cortex [Anitschkov & Poskalenko (8)].

Soviet pharmacologists eagerly search for new sedatives and investigate their action. By using various methods, chiefly that of conditioned reflexes, it was discovered that certain aglycones of cardiac glycosides, such as strophanthidin and erysimidin, possessed these properties in a degree fully justifying their application in clinical practice [Asratian (9)]. It was furthermore shown that several anticonvulsants, such as diphenylhydantoin and trimethadione, act as vestibular tranquilizers and may be used in the treatment of Ménière's disease [Malyghina (18); Milstein (21); Yaroslavski (31)]. Certain amines of 2-phenyl-1,3-indandione were found to possess a tranquilizing and anticonvulsant effect, and the structure-activity relationship was investigated [Belenki and co-workers (10)]. Synthetic alkyl derivatives of diamide and imidazoldicarboxylic acid were shown to possess a depressing effect upon the conditioned reflexes antagonistic to caffeine and a stimulating one upon the subcortical region [Anichkov & Borodkin (7)]. The action of analgesics and several other drugs upon the efferent system of the reticular formation was investigated by Valdman (28).

The study of another branch of nervous system pharmacology actively pursued in Soviet Russia is that of the ganglia and neuromuscular synapses. Thanks to the work of Mashkovski and co-workers (19), use is made in this country of a number of natural alkaloids such as pachycarpine (an optic isomer of sparteine), obtained from *Sophora pachycarpus,* and sphaerophysine, extracted from *Spaerophysalsula.* Both alkaloids are bis-tertiary bases and possess a "mild" ganglion-blocking action, do not lose their properties on oral administration, and are used in the same way as oxytocic drugs.

In the search for new ganglion-blocking agents the Latvian and Ukrainian pharmacologists, headed respectively by Professor Belenki [Schuster (25)] and Professor Tcherkes [Dombrovskaia and co-workers (12)], carried out researches in the field of structure-action relationships of gangliolytics. Tcherkes' group has shown the important role played by anions in the gangliolytic action of salts of bis-quaternary bases. It was ascertained that hexamethonium benzene sulfonate was a more powerful gangliolytic and was less toxic than the usual iodide. In the U.S.S.R., hexamethonium dibenzene sulfonate, known as benzohexonium, is widely used as a gangliolytic. Kharkevitch (17) showed that the best criterion for estimating gangliolytic depressive action was the reduction of functional lability of ganglionic neurons. All gangliolytics further Vvedenski's inhibition following irritation of preganglionic fibers by high-frequency impulses. This decrease in functional lability appears much earlier than the change in amplitude of postganglionic potentials. Obviously, the reaction of ganglionic cells to gangliolytics is not restricted to a decrease of sensitivity to mediators. Research carried out by the same author has shown that gangliolytics not only retard impulse transmission in ganglionic synapses, but actually exert a definite influence upon the subsequent processes, preventing post-tetanic, impulsive after-discharges.

Mitrofanov (22) showed that ephedrin, in contrast with gangliolytics, heightened the lability of ganglionic neurons and shortened the period of hexamethonium- and tetraethylammonium-induced blockade. Vysotskaia (30) studied the influence of gangliolytics upon ganglion metabolism. It was found that hexamethonium, a ganglion-blocking agent acting as a competitive antagonist to acetylcholine, did not lower the level of adenosinetriphosphate and of creatine-phosphate in the cervical ganglion tissues, whereas a depolarizing agent such as nicotine, by blocking the ganglia, caused a fall of adenosinetriphosphate and a rise in inorganic phosphate. For this reason, nicotine-induced blockade is impaired by adenosinetriphosphate, although the same substance exerts no action upon hexamethonium blockade. While studying gangliolytics, Soviet pharmacologists investigated their action not only upon the function of autonomically innervated organs, but also their action upon the tissue metabolism of these organs [Anichkov (3) and Zavodskaya (33)]. It was found that gangliolytics depress protein resynthesis in the gastric and intestinal mucosa, as estimated by the in-

corporation rate of labelled methionine. On the other hand, they bring this resynthetizing process nearly to normal level after depression by reflexes induced by intense irritation. Normalization of trophic processes is considered by Soviet pharmacologists to be the main factor in the preventive and curative effect of gangliolytics in cases of reflex gastric wall distrophy underlying peptic ulcer.

Because plants containing curarine or any previously known curariform alkaloids do not grow in the U.S.S.R., a search for curariform drugs has been a very urgent problem in this country. A systematic study of the alkaloids extracted by the late academician, A. P. Orekhov, led to the discovery in certain species of *Delphinium* of alkaloids with curare-like action (delcimine, elatine, condemarine, mellitine) [Mashkovski (19)]. These are tertiary amines; they are orally active and have been adopted mainly in neurological clinic work for depressing muscular tone. The drug evolved by VNIKhFI under the name of diplacin is synthesized using a natural alkaloid as primary material. It is a dichloride 1,3-di-(β-platineciniumethoxy)-benzene [Mashkovski (19)]. Another Soviet synthetic curariform drug is paramione (diiodide-mezo-3,4-diphenyl-hexane-n,n'-bis-trimethylammonium) brought forth by the Institute of Experimental Medicine [Torf and co-wokers (27)]. Conclusions as to the dependance of curare-like activity of bis-ammonium compounds upon the rigidity of their molecules were reported in 1958 at the Symposium on Curariform Drugs in Venice [Anitchkov & Khromov-Borissov (4)]. The influence of cholinolytics, narcotics, anticholinesterases, and of poisons impairing conjugate phosphorylization, upon the pathological stimulation elicited in neuromuscular synapses by the action of guanidine and other poisons was the subject of an investigation by Karasik (14). The same author (13) availed himself of the so-called "Holden's paradox," apparent in the reaction of hemoglobin with O_2 and CO_2 for the interpretation of the competitive antagonism existing between acetylcholine and synaptic poisons.

The pharmacology of the cardiovascular system is another province of traditional scientific activity in the Soviet Union. This was the sphere of study of N. P. Kravkov, one of the founders of Russian pharmacology who proposed the isolated rabbit-ear method and studied on a large scale the pharmacology of vessels by perfusing isolated animal and human organs. During recent years, extensive work on cardiac glycosides has been performed by the Kharkov Chęmo-Pharmaceutical Institute [Angarskaja and co-workers (1)]. A systematic investigation of various plants has led to the extraction of hitherto unknown cardiac glycosides, such as syretoxin from *Syrena angustifolia* and homphotine from *Homphocarpes fruticosces.* According to their pharmacological properties, these glycosides may be classed with the strophanthine group, although the data obtained by the Kharkov pharmacologists show that homphotine differs from strophanthine in that it is able to dilate coronary vessels. Experimental myocarditis was investigated by Vedeneieva (29), who obtained lesions of the myocardium by

massive doses of epinephrine and norepinephrine, as well as by intense irritation of the ganglion stellatum. Sympatholytics prevent this myocarditis. Extensive work has been devoted to searching for vasodilators, especially coronary vasodilators. Kaverina (15) undertook a systematic study of the influence of drugs upon the lumen of coronary vessels. Most promising among the newly proposed preparations is chloracizine (10,β-diethylaminopropionyl-2-chloro-phenothiazine) a drug evolved by the Pharmacological Institute. Chloracizine increases coronary blood flow, preventing the pituitrin-induced spasm of the vessels. This process is not accompanied by a fall in blood pressure and does not increase the cardiac oxygen consumption. In experimental infarction of the myocardium, chloracizine stimulates the formation of collaterals [Zakusoff (32)]. Kharkov phytochemists and pharmacologists [Angarskaja & Chadjai (2)] have been engaged in the search for vasodilators of plant origin. Preparations obtained from the seeds of the common carrot (diucarine), parsnip (pastivacine), and fennel (anethine) were proposed as such vasodilators. Diucarine and anethine, as compared with other preparations of the same type, have proved to possess low toxicity associated with relatively high spasmolytic activity.

The individual substances extracted from these plants have shown that some furocoumarins and flavones possess coronary vasodilator properties. Experimental therapy of vascular diseases is represented by works on the pharmacotherapy of experimental cholesterol atherosclerosis according to the well-known method of the eminent pathologist, N. N. Anichkov. Among the numerous Soviet works in this field must be noted the investigations of the last few years devoted to the influence upon cholesterolemia and cholesterol aortal atheromatosis of the gangliolytic hexamethonium [Novikova (24)], the spasmolytic dibazol [Kharauzov & Novikova (16)], and the saponines of *Dioscorea* and *Polemonium coeruleum* [Sokolova (26)]. These substances bring about a certain amelioration in the course of cholesterolemia and cholesterol atheromatosis.

The present writer has not been able to mention in such a brief article all the work done by Soviet pharmacologists even in the restricted field reviewed. All he could do was to show, by a few examples, how Soviet Pharmacologists, on a basis of exact data, have endeavored to serve in a practical way the cause of public health.

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* English translation will be announced in *Technical Translations*, issued by the Office of Technical Services, U. S. Department of Commerce, and will be made available by the Photoduplication Service, Library of Congress, and by the Translation Center at the John Crerar Library, Chicago, Illinois.

MECHANISMS OF DRUG ABSORPTION AND DISTRIBUTION[1,2]

By Lewis S. Schanker

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During the past three to four years, considerable progress has been made toward defining the mechanisms of drug absorption and distribution. Perhaps one of the reasons for this rapid advance has been the adoption of the view that the mechanisms of absorption and distribution of foreign organic compounds may be considerably simpler than those of the natural cell substrates. This idea has developed from the numerous studies of membrane permeability of the past 60 years. The classical work of Overton (1) and of Collander & Bärlund (2) on the permeability of plant and animal cells to organic compounds led to the view that the cell boundary is essentially a lipid-like layer interspersed with small aqueous channels; nonpolar substances would penetrate the membrane by dissolving in the lipoid phase, and polar molecules would penetrate only if they were small enough to diffuse through the open channels. Later workers recognized that the cell membrane also possessed specialized processes for transporting lipid-insoluble substrates required by the cell (3). For example, certain sugars and amino acids were found to cross the cell boundary by processes which differed from simple diffusion in that they exhibited specificity, saturability, and a requirement for energy. Although the voluminous literature dealing with specialized transport processes tended to relegate to the background the lipoid sieve theory of membrane permeability, recent studies of the absorption and distribution of drugs have shown the great importance of this early concept. The gastrointestinal epithelium, the renal tubular epithelium, the blood-brain and blood-cerebrospinal fluid boundaries, and the boundaries of various tissue cells behave as lipid-like barriers to the passage of many foreign organic compounds. Numerous drugs diffuse across these boundaries as lipid-soluble, nonionized molecules, and the rates of transfer are, in general, related to the relative lipid/water partition coefficients of the molecules. The almost lipid-insoluble, ionized forms of drugs diffuse across these boundaries with relatively great difficulty.

However, the lipoid membrane thesis does not explain completely the passage of drugs across the boundaries of mammalian cells. Specialized transport processes appear to be responsible for the rapid cellular transfer of certain foreign organic ions and polar molecules.

This article reviews a number of investigations of the past few years which attempt to define the mechanisms of drug absorption and distribution.

[1] The survey of literature pertaining to this review was concluded in May, 1960.

[2] Abbreviations used in this chapter include: CSF (cerebrospinal fluid); EDTA (ethylenediamine-tetraacetic acid).

PASSAGE OF DRUGS ACROSS THE GASTROINTESTINAL EPITHELIUM

Distribution of drugs between plasma and gastric juice.—A study by Shore *et al.* (4) of the distribution of drugs between plasma and gastric juice has supplied strong evidence that drugs cross the gastric epithelium in their nonionized form, and that the ionized form penetrates very slowly, if at all. In the experiments, various acidic and basic drugs were administered intravenously to dogs with Heidenhain gastric pouches, and the concentrations of drug in gastric juice and plasma measured. At the steady state, basic drugs appeared in gastric juice in concentrations ranging

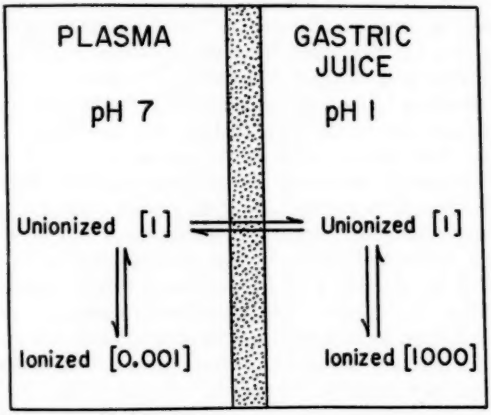

FIG. 1. Theoretical distribution of an organic base, pKa 4, between plasma and gastric juice, assuming that the fluids are separated by a boundary permeable only to the un-ionized drug molecule.

from one to 40 times that of plasma. In contrast, acidic drugs appeared in gastric juice in low concentrations which ranged from zero to six-tenths that of plasma. The results were explained in terms of a model system in which gastric juice is separated from plasma by a barrier permeable only to the nonionized form of a weak electrolyte (Fig. 1). At the steady state, the concentrations of nonionized drug in plasma and gastric juice are the same (correcting for the degree of plasma binding), but the concentrations of the ionized form are unequal because of the difference in hydrogen-ion concentration of the two fluids. Accordingly, the total concentration of drug (ionized plus nonionized) on both sides of the gastric mucosa is a function of the pH of the two fluids and the dissociation constant of the drug. From this relationship, it can be readily calculated that a basic drug will be more concentrated in gastric juice than in plasma, and an acidic drug will be more concentrated in plasma than in gastric juice. The gastric juice/plasma concentration ratios observed in this study were generally in close agreement with the calculated ratios; however the maximum ratio of 40, observed

for a number of basic compounds, was considerably less than the values calculated for these compounds. The apparent inconsistency was resolved when it was shown that the ratio of 40 was a limiting value imposed by the rate of gastric mucosal blood flow.

This interpretation of the distribution of drugs across the gastric mucosa is consistent with the observations of a number of earlier workers that basic dyes, administered parenterally, appear in the gastric lumen, whereas acidic dyes do not. The hypothesis is also supported by the recent findings of Zawoiski *et al.* (5), who studied the gastric secretion of mecamylamine in the dog. This organic base readily passes from plasma into the gastric lumen where it becomes concentrated to an extent dependent on the pH of the gastric juice—the lower the gastric pH, the higher the gastric juice/plasma concentration ratio.

The establishment of concentration gradients of weak electrolytes across the gastric epithelium is, as shown later in this article, but one example of a type of drug distribution observed with many biologic membranes. All that is required for such a distribution is that the membrane have a preferential permeability to the nonionized form of the weak electrolyte, and that the fluids bathing either side of the membrane have different hydrogen ion concentrations. The process is physical in nature and does not display the characteristics of a specialized transport system; there is, however, an indirect expenditure of energy by the organism in maintaining the pH differential across the membrane.

Absorption of drugs from the stomach.—Most of the recent studies of the mechanism of gastrointestinal drug absorption, as well as some of the earlier papers on this topic, have been reviewed by the author (6).

Schanker *et al.* (7) have shown that a variety of drugs are absorbed directly from the rat stomach by simple diffusion of the nonionized drug moiety. Of the large number of compounds studied, ready absorption was observed for all of the acidic drugs except the highly ionized sulfonic acids. In contrast, none of the basic compounds were absorbed except those so weakly basic that they are partially nonionized in the gastric contents.

Additional evidence that it is mainly the nonionized form of a drug which is absorbed was obtained by changing the degree of ionization of drugs by raising the pH of the stomach contents. Basic compounds, which become more un-ionized at higher pH values, were more readily absorbed from the alkaline medium: Conversely, acidic compounds, which become more ionized at higher pH values, were less readily absorbed. Evidence of the passive nature of the absorption process was supplied by the observations that various drugs did not interfere with the absorption of other drugs, and that the amount of drug absorbed was directly proportional to the concentration within the stomach.

An indication that lipid solubility is the physical property governing the passage of uncharged molecules across the gastric epithelium was provided by a study of three barbiturates with similar pKa values. These compounds were absorbed at rates roughly proportional to the lipid/water partition co-

efficients of their nonionized forms. For example, thiopental was absorbed very rapidly, secobarbital less rapidly, and barbital relatively slowly.

Hogben *et al.* (8) reported that the pattern of absorption from the stomach of man was the same as in the rat (7). Acidic drugs like salicylic acid, acetylsalicylic acid, thiopental, and secobarbital were readily absorbed. Basic compounds like quinine, ephedrine, and aminopyrine were not absorbed. Of considerable interest was the observation that thiopental and the two salicylates were absorbed more rapidly than ethyl alcohol; heretofore ethanol had often been considered the rather unique example of a drug absorbed from the stomach.

Absorption of drugs from the small intestine.—Schanker *et al.* (9) have found that the epithelial lining of the intestine, like that of the stomach, allows the ready penetration of undissociated drug molecules but impedes the passage of ionized moieties. In experiments with rats, the entire small intestine was perfused with a drug solution, and the extent of absorption estimated from the difference in the concentration entering and leaving the intestine. A relation between the degree of ionization and the rate of absorption of drugs was revealed: the weaker acids and bases were readily absorbed; stronger, highly ionized acids and bases were more slowly absorbed; and the completely ionized quaternary ammonium compounds and sulfonic acids were hardly absorbed at all. It was pointed out that the failure to observe any measureable absorption for some of the very highly ionized compounds was not inconsistent with the slow but definite absorption of these substances known to occur in therapeutics; in the perfusion experiments, the drug solution passed through the intestine in only seven minutes, contrasted with the several hours that a drug remains in the intestine when used therapeutically.

The direct proportionality between the amount of salicylic acid or aniline absorbed and the concentration of drug within the intestine suggested that absorption occurred by simple diffusion rather than by a specialized transport process which would be expected to become saturated at the higher drug concentrations. Additional evidence of the passive nature of the absorption process was the failure of various drugs to alter the rates of absorption of other drugs.

Since the rates of intestinal absorption of drugs were related to the proportion of lipid-soluble, undissociated drug molecules and not to the molecular weight of the compounds, it was suggested that the main pathway of absorption is through the lipoid areas of the intestinal boundary rather than through small aqueous channels. In support of this view, many lipid-soluble drugs of high molecular weight were absorbed more rapidly than small, lipid-insoluble molecules like urea and D_2O.

Hogben *et al.* (10) studied the effect of changes of intestinal pH on the absorption of a number of weak organic acids and bases in the rat. It was shown that the rate of absorption varied with the proportion of drug present as nonionized molecules. For example, raising the intestinal pH increased

the absorption of bases and decreased the absorption of acids; moreover, compounds which remained essentially undissociated at the various hydrogen-ion concentrations tested showed no change in their rate of absorption.

These investigators also measured the distribution of various drugs between plasma and the intestinal lumen. In the experiments, the rat intestine was perfused with a solution of the drug, and the animal also received the drug intravenously. At the steady state, when there was no net passage of drug from intestine to plasma or from plasma to intestine, the gut/plasma concentration ratios approached, but did not equal the ratios calculated for a system in which plasma (pH 7.4) and intestinal contents (pH 6.6) are separated by a lipoid membrane. The results suggested that the effective pH at the site of absorption might be different from the pH of the intestinal contents. On calculating the effective pH of the intestine from the observed gut/plasma concentration ratios, a value of 5.3 was obtained. A zone with a pH of 5.3, possibly located at the surface of the intestinal epithelial boundary, was shown to be quantitatively consistent with the pattern of drug absorption in the rat intestine.[3]

Evidence that lipid solubility is the physical property that determines the rate of passage of uncharged molecules across the intestinal epithelium was provided by the observation that the rates of absorption of a large number of weak acids and bases were roughly parallel to the lipid/water partition ratios of the nonionized drug molecules.

Intestinal absorption of quaternary ammonium ions.—Although the intestinal absorption of weak organic electrolytes can be explained in terms of simple diffusion of uncharged molecules across a lipoid boundary, the question remains how organic ions are absorbed. The rates at which organic anions and cations cross the intestinal epithelium are extremely slow compared with the rates of passage of most uncharged molecules (9, 10). Nevertheless, it is well known that ions like the quaternary ammonium compounds are absorbed to a significant extent when administered therapeutically. For example, it has been estimated that 5 to 10 per cent of an oral dose of hexamethonium is absorbed in man (13, 14, 15).

Levine *et al.* (16, 17) studied the absorption of a number of quaternary ammonium ions by measuring their rates of disappearance from loops of the rat small intestine. It was observed that in general the rate of absorption declined markedly with time. For example, several quaternary ammonium compounds were absorbed to the extent of about 15 per cent in four hours, but most of the absorption took place during the first hour. An exception is penthienate, which was continuously absorbed throughout five hours [Levine & Clark (18)]. It was suggested that the poor absorption of these drugs is attributable to the formation of nonabsorbable complexes with mucin. This view was supported by the observation that adding mucin to the intestinal loop depressed the degree of absorption of benzomethamine (16).

[3] The phenomenon of the difference in pH between a biologic surface and the solution which bathes it has been discussed by Albert (11) and by Caldwell (12).

More recently Levine (19) reported that, although intestinal mucus can form nonabsorbable complexes with quaternary ammonium compounds, removal of the mucus by washing the intestine results in a decrease rather than an increase in the absorption of these ions. It was suggested that the mucus might contain some component capable of forming an absorbable complex with these drugs. However a variety of constituents of normal intestinal contents failed to augment the absorption of benzomethamine. For example, adenylic acid, glucose-1-phosphate, chondroitin sulfate, and hyaluronic acid were found to inhibit absorption; amino acids, saturated fatty acids, bile, bile salts, sorbitol, glucuronic acid, and mucic acid had no effect on the absorption of benzomethamine.

The available information raises a number of questions about the mechanism of absorption of quaternary ammonium ions. Does intestinal mucus promote as well as inhibit the absorption of these cations? Does the binding of quaternary ammonium compounds to mucus have something to do with the process by which these drugs cross the intestinal epithelium, or does it result only in a lowering of the concentration of diffusable drug? If quaternary ammonium ions are absorbed as complexes, what is the nature of the complex, and what happens to it on reaching the bloodstream? Whether these compounds are absorbed in the unchanged form or as complexes, do they cross the intestinal epithelium by simple diffusion or by specialized transport processes analogous to those which transport certain inorganic cations? A clue to the answer of the last question might be obtained from a thorough study of the kinetics of the absorption process.

Intestinal absorption of tetracycline.—Tetracycline is a highly ionized drug which, like the quaternary ammonium compounds, is only partially absorbed from the gastrointestinal tract when administered therapeutically [Maynard *et al.* (20)]. During the past few years, reports from several laboratories have claimed that the absorption of tetracycline in man is significantly enhanced when the antibiotic is administered in capsules containing citric acid, sodium hexametaphosphate, or glucosamine. Numerous comparisons of the effectiveness of the various adjuvants have been made, and conflicting results reported. For example, various investigators found different adjuvants to be superior to the others tested, and some investigators found that the adjuvants had no effect on the absorption of tetracycline.

In reviewing a number of these reports, Finland (21) concluded that several of the claims of enhanced absorption were not supported by the experimental data. He also pointed out that much of the conflicting data was the result of failure to control the amount of calcium salts in the various capsule preparations. Dicalcium phosphate has been shown to depress markedly the absorption of tetracycline [Dearborn *et al.* (22)]; the same salt is commonly used as a filler in capsule preparations on the assumption that it is inert. Finland (21) and Kunin *et al.* (23) have concluded that there is little or no difference in the blood levels of tetracycline achieved in clinical practice whether the antibiotic is administered as the

pure (calcium-free) hydrochloride salt or in combination with various adjuvants.

From a study in rats, in which the blood level of tetracycline was taken as a measure of the degree of absorption, Dearborn *et al.* (22) concluded that both calcium ion and food interfered with the absorption of tetracycline, and that citric acid and sodium metaphosphate partially reversed the effect. The authors suggested that citric acid and sodium metaphosphate facilitate the absorption of tetracycline by complexing with calcium and other multivalent metal ions which are present in the gastrointestinal tract and which form complexes with tetracycline.

Although the results of the above studies are interesting and have important implications in the therapeutic use of tetracycline, they give no indication of the process by which this drug is absorbed. The work of Pindell *et al.* (24) has supplied the only clear information regarding the mechanism of absorption of tetracycline. These workers measured directly the extent and rate of absorption of the drug from loops of the dog small intestine. Only about 3 per cent of an administered dose of tetracycline was absorbed in 1.5 hours, and the rate of absorption was constant throughout this period. The amount of tetracycline absorbed was directly proportional to the concentration over a tenfold range, and it was concluded that absorption occurs by passive diffusion.

Considering that tetracycline exists largely as a zwitterion at neutral pH (25), it would be interesting to know whether this drug is absorbed predominantly as the zwitterion, anion, cation, or neutral molecule.

Intestinal absorption of large molecules.—Loomis (26) has reported that heparin, a polysaccharide ordinarily not absorbed from the gastrointestinal tract, can be absorbed to a small extent when the intestinal contents are made acidic. In the experiments, solutions of heparin were placed in duodenal loops of the dog, and the degree of absorption was estimated from changes in the clotting time of blood as well as from the amount of drug recovered from the intestine after five to seven hours. An anticoagulant effect was noted when the drug was administered in a citrate-phosphate solution of pH 4; in contrast, no absorption occurred from a similar solution of pH 8 or from physiological saline solution. Since the anticoagulant effect lasted only three hours, and only about 10 per cent of the dose was absorbed, it appeared that absorption ceased as the intestinal pH rose from 4 to 6.5. It was suggested that the absorption of heparin from a solution of low pH might be attributed to the lowered degree of ionization of the molecule's carboxyl groups.

Windsor & Cronheim (27) have found that heparin and a synthetic heparinoid, sulfopolyglucin, are absorbed from the gastrointestinal tract of the dog and rat when administered with certain salts of ethylenediaminetetraacetic acid (EDTA). The sodium, potassium, and ammonium salts of EDTA were effective, but the calcium and magnesium salts were not. It was suggested that the alkali salts of EDTA facilitate the absorption of heparin and sulfopolyglucin by forming chelates with calcium and mag-

nesium ions in the intestinal lumen. In support of this view, the oral administration of calcium ion blocked the enhancement of absorption produced by alkali salts of EDTA.

Trace amounts of polypeptides and proteins are known to be absorbed from the gastrointestinal tract. For example, Laskowski *et al.* (28) estimated that in six hours somewhat less than 3 per cent of a dose of insulin was absorbed from a loop of the rat small intestine when the drug was administered together with a pancreatic inhibitor of trypsin and chymotrypsin. When insulin was not protected from the actions of proteolytic enzymes, absorption did not occur. From similar experiments in the rat, Danforth & Moore (29) concluded that insulin was absorbed to the extent of about 0.05 per cent in three hours when administered with diisopropylfluorophosphate, another inhibitor of the actions of proteolytic enzymes.

The toxicity of the ingested exotoxin of *Clostridium botulinum* and the allergic response to ingested proteins of food are familiar examples of the intestinal absorption of protein molecules. Lamanna (30) has recently shown that the diphtheria and tetanus toxins, as well as the botulinus toxin, are absorbed from the gastrointestinal tract of mice. Absorption could be demonstrated only when huge amounts of the toxins were administered, the oral LD_{50} values being hundreds of thousands of times greater than the intraperitoneal LD_{50} values. It was suggested that protein molecules are absorbed by passing through imperfections in the intestinal boundary. Pinocytosis ("cell drinking") has also been suggested as a process by which large molecules may penetrate cell boundaries [Leake & Pomerat (31)].

Absorption of drugs from the colon.—Schanker (32) found that the pattern of drug absorption in the rat colon was very similar to that in the small intestine. Weak acids and bases were in general readily absorbed; stronger, more highly ionized acids and bases were more slowly absorbed; and the completely ionized quaternary ammonium compounds and sulfonic acids were very slowly absorbed. Moreover, the absorption of weak acids and bases was favored by a change in colonic pH which increased the proportion of drug in the nonionized form. The lipid solubility of drugs was shown to be an important physical property in governing the rate of absorption. For example nine barbiturates with similar pKa values were absorbed at rates roughly proportional to the chloroform/water partition ratios of the nonionized drug molecules.

Active transport across the intestinal epithelium.—Although the intestinal absorption of numerous drugs and other foreign organic compounds may be explained in terms of simple diffusion across a lipid-like boundary, it is also clear that certain natural substrates are absorbed by specialized active transport processes. It has long been recognized that the small intestine possesses two independent active transfer processes for organic compounds: one for monosaccharides, and one for amino acids. A third such process, which actively transports the pyrimidines thymine and uracil, has recently been described by Schanker & Tocco (33).

The active absorption mechanisms are poorly understood and can be

described only in terms of the characteristics which distinguish them from simple diffusion. These include: transport of the solute against a concentration gradient; saturation of the transport mechanism when the concentration of solute is raised high enough; specificity of the process for a certain molecular structure; competition between two solutes for the same transfer mechanism; and inhibition of the transport process by substances which interfere with cell metabolism.

There is evidence that a drug can be transported by one of the specialized processes if its chemical structure is similar enough to that of the normal substrate. For example, Wilson & Landau (34) have shown that several foreign sugars, structurally similar to glucose, are actively transferred by the monosaccharide transport mechanism of the small intestine. Further, the foreign compounds, 5-fluorouracil and 5-bromouracil, are actively transported across the intestinal epithelium by the pyrimidine transport system (35).

Absorption of Drugs Through the Skin

The percutaneous absorption of drugs has been the subject of several recent reviews (36 to 39). Although it has long been recognized that in general lipid-soluble molecules penetrate the skin much more readily than do lipid-insoluble molecules and ions, conclusive evidence in support of this view has been obtained only recently. In a study of the permeability of excised rabbit skin to a number of nonelectrolytes, Treherne (40) clearly demonstrated the lipoid character of the epidermal barrier. It was shown that various organic substances diffuse across whole skin at rates roughly proportional to the ether/water partition coefficients of the compounds. It was concluded that the lipid-like barrier of the skin is located within the epidermal layer, since the dermis is freely permeable to many solutes and displays the characteristics of a highly porous membrane.

Ions and lipid-insoluble molecules, which penetrate the skin very slowly, appear to by-pass the epidermal barrier by diffusing through the walls of the hair follicles and sebaceous glands [Malkinson (36)].

Passage of Drugs Across the Renal Tubular Epithelium

It is beyond the scope of this article to present a detailed account of studies of the mechanisms of renal drug excretion. From several comprehensive reviews that have appeared recently, it is possible to make the following generalizations.

The renal tubular epithelium appears to have a dual character regarding the transfer of foreign organic compounds: it behaves as a lipoid boundary which allows the ready passage of nonionized, lipid-soluble molecules, and it has specialized processes for the transport of many organic ions.

Drugs of high lipid solubility like thiopental do not appear in the urine in appreciable amounts, since nearly all of the drug molecules filtered at the glomerulus return to the bloodstream by diffusing across the lipid-like

boundary of the renal tubule (41, 42, 43). Conversely, compounds of relatively low lipid solubility like barbital are more readily excreted in the urine because they are only partially reabsorbed in the tubule.

The changes in the rate of urinary excretion of weak acids and bases that result from changes in the pH of tubular fluid are consistent with the view that the tubular epithelium is selectively permeable to the nonionized, lipid-soluble form of drugs. Thus the distribution of weak acids and bases between tubular urine and plasma can be explained by the same principles outlined previously in this article for the distribution of drugs between gastric juice and plasma. For example, when the tubular urine is more alkaline than plasma, weak bases become less concentrated in urine than in plasma, and, as a result, are slowly excreted; when the urine is acidic, weak bases become concentrated in the urine and are rapidly excreted [Orloff & Berliner (44)]. Moreover, weak acids are readily excreted in an alkaline urine, and more slowly excreted in an acidic urine [Waddell & Butler (45, 46)]. In recent reviews, Milne *et al.* (47) and Peters (48) evaluate the importance of passive tubular transfer of nonionized molecules in the urinary excretion of weak acids and bases.

The renal tubular epithelium appears to possess at least two specialized transport processes: one for the secretion of strong organic acids, and one for the secretion of strong organic bases. Many of the compounds shown to be excreted by these processes are completely ionized at pH 7.4 and would not readily diffuse across a lipoid membrane. Numerous studies have indicated that these substances are transported across the renal tubule against large concentration gradients, that there is competition for transport among various acidic compounds, and that there is competition for transport among various basic compounds. These observations have led to the conclusion that many strong organic acids and bases cross the renal tubule by active transport processes. Peters (48) has recently reviewed the renal excretion of organic bases, and Sperber (49), that of organic acids; Taggart (50) presents an interesting discussion of the renal transport processes.

The mechanisms of urinary excretion of weak acids and bases and of strong acids and bases are often considered to be essentially different, the former involving passive diffusion, and the latter active transport. But surely the kidney tubule cannot distinguish a weak acid from a strong acid, or a weak base from a strong base; more likely, the tubular epithelium can only distinguish an ion from an uncharged molecule. Accordingly, one might expect the ionized form of a weak acid or base to be actively transported, provided that it possessed the structural characteristics required by the transport system. Evidence of this has been supplied by a recent study of the renal excretion of salicylic acid [Weiner *et al.* (51)]. It was shown that the salicylate ion is secreted into the tubular fluid by an active transport process resembling the one which transports *p*-aminohippurate. The tubular reabsorption of salicylic acid appears to occur by passive diffusion of the unionized drug molecule and is governed by the difference in pH between tubular urine and plasma.

A similar three-phase excretion process—glomerular filtration, active tubular secretion, and passive tubular reabsorption—has not yet been demonstrated for weak organic bases, although it was proposed a number of years ago by Jailer *et al.* (52) and is strongly suggested by the recent work of Peters and co-workers (48).

Passage of Drugs into Bile

The mechanisms of biliary excretion of drugs are poorly understood. Many substances appear in bile in a concentration similar to that of plasma. On the other hand, a number of highly ionized organic acids are secreted into bile in very high concentrations, suggesting the presence of an active transport system analogous to the one of the kidney. Studies of the passage of drugs and other substances into bile have been reviewed by Sperber (49) and by Brauer (53).

Schanker & Hogben (54) have recently investigated the biliary excretion of some lipid-insoluble molecules in the rat. The appearance in bile of such large molecules as inulin and sucrose suggests that the boundary between the blood and the bile is a highly porous structure. The observation that sucrose and mannitol invade the intracellular water of liver, mannitol rapidly equilibrating with all of the hepatic water, implies that the pores are located in the membrane of the hepatic parenchymal cell. This cell would thus appear to be unique among animal cells with its unusual permeability to solutes generally confined to the extracellular compartment of body tissues.

Passage of Drugs into the Central Nervous System

The view that the blood-brain and blood-cerebrospinal fluid barriers behave as lipoid membranes toward foreign organic compounds [Davson (55, 56); Brodie & Hogben (41)] has received strong support from several recent studies. Mayer *et al.* (57) showed that a variety of drugs penetrate the brain and cerebrospinal fluid (CSF) of rabbits at rates roughly parallel to the lipid/water partition coefficient of the drugs at pH 7.4. It was noted for several compounds that the rates of entry into brain and CSF were very similar and that the transfer process was best described in terms of simple diffusion.

Mark *et al.* (58, 59) have demonstrated a relation between the rate of passage into brain and the oil/water partition ratio at pH 7.4 of several barbiturates. Thiopental, with its very high lipid solubility, entered the brain of dogs so rapidly that the rate was probably limited only by the rate of cerebral blood flow.

A study by Rall *et al.* (60) of the distribution of drugs between the CSF and plasma of dogs has indicated that the blood-CSF boundary, like other biologic membranes, is preferentially permeable to the nonionized form of weak organic acids and bases. Antipyrine, *p*-aminobenzoic acid, and several sulfonamides were shown to attain steady-state CSF/plasma concentration ratios which approximated those calculated for a lipoid membrane separating solutions of the pH of blood and CSF. Furthermore, when the pH gradient between blood and CSF was altered by changing the pH of plasma,

compounds whose degree of ionization was significantly affected attained new CSF/plasma concentration ratios which approached the predicted ratios; drugs like antipyrine and sulfanilamide, which remained essentially undissociated at the various pH values, showed no change from their normal distribution ratio of 1.0. The distribution between CSF and plasma of ammonia [Stabenau *et al.* (61)] and barbital [Brodie *et al.* (62)] has also been shown to be a function of the pH of the two fluids and the dissociation constant of the compound.

More direct evidence that the blood-brain and blood-CSF barriers are highly resistant to the entry of foreign organic ions has been supplied by studies of completely ionized substances like the quaternary ammonium compounds and sulfonic acids. These cations and anions penetrate the brain and CSF at extremely slow rates [Brodie *et al.* (62); Mayer & Bain (63); Rall & Zubrod (64)].

Brodie *et al.* (62) have evaluated the factors of lipid solubility and the degree of ionization in the passage of drugs into cerebrospinal fluid. Fourteen compounds of diverse structures and physical properties were found to diffuse passively from plasma into the CSF of dogs at widely different rates. Lipid solubility was shown to be the rate-limiting factor with drugs that are mainly undissociated in plasma; these compounds penetrated the blood-CSF barrier at rates roughly related to the lipid/water partition coefficient of the nonionized molecules. The degree of ionization was shown to be the rate-limiting factor with compounds that are highly ionized in plasma; these drugs entered the CSF at rates roughly parallel to the proportion of drug nonionized at pH 7.4. It was suggested that although both lipid solubility and the degree of ionization are important in governing the transfer of drugs into CSF, lipid solubility is probably the dominant characteristic since the relevance of the degree of ionization is probably a consequence of the lipid insolubility of organic ions.

Although the mechanism of entry of drugs into the central nervous system appears to be fairly well understood, the same cannot be said for the mechanism of exit. Mayer *et al.* (65) reported that a number of drugs rapidly disappeared from the CSF of rabbits after intracisternal injection. Although compounds with high lipid solubilities left the CSF somewhat more rapidly than those with low lipid solubilities, the difference in the rates of exit was slight when contrasted with the widely diverse rates at which the same compounds enter the cerebrospinal fluid. It was suggested that the chief mode of exit from the CSF for many drugs may be diffusion from the subarachnoid space through the arachnoid villi into the bloodstream; lipid-soluble drugs might also leave the CSF to some extent by penetrating the blood-CSF and blood-brain barriers in the reverse direction. The observation that drugs may leave the CSF quite rapidly regardless of their rate of entry would explain why a slowly entering compound like sulfanilic acid (60) never attains a CSF/plasma ratio of unity.

A recent study by Pappenheimer *et al.* (66) indicates that certain compounds may leave the CSF by a process other than simple diffusion. For ex-

ample iodopyracet (Diodrast), phenol red, and *p*-aminohippuric acid were shown to be actively transported from CSF to blood in both the cat and goat. The drugs appear to be secreted from a region somewhere between the aqueduct of Sylvius and the cisterna magna; secretion does not occur from the lateral ventricles, the third ventricle, or from the cortical subarachnoid spaces. At low drug concentrations, at which the transport mechanism is not saturated, the rate of active secretion from CSF is about 10 times the rate of passive transfer.

Passage of Drugs into Tissue Cells

Effect of plasma pH on drug distribution.—In a study of the distribution of phenobarbital in dogs, Waddell & Butler (45) found that the partition of the drug between plasma and tissues could be altered reversibly by varying the plasma pH. When the plasma pH was lowered, the plasma drug level decreased and the tissue levels (brain, fat, liver, and muscle) increased; conversely, raising the plasma pH resulted in an increase in the plasma level and a decrease in the tissue levels. The results were consistent with the assumption that cell membranes are preferentially permeable to the nonionized form of phenobarbital and that the drug distributes between the intracellular and extracellular fluids according to the difference in pH of the fluids.

Waddell & Butler (67) have recently used the pH-dependent distribution of a weak acid, 5,5-dimethyl-2,4-oxazolidinedione (DMO) to estimate the intracellular pH of skeletal muscle in the dog. The value of 7.0 obtained with this compound is in close agreement with earlier reported values based on the distribution of carbon dioxide.

Effect of plasma protein binding on drug distribution.—Numerous studies have shown that drugs bound to plasma proteins cannot cross body membranes. For example, it is only the unbound fraction of drug in plasma which penetrates the blood-brain and blood-cerebrospinal fluid boundaries (57, 60, 62), the renal tubular epithelium (48, 49), or the gastrointestinal epithelium (4, 10, 32).

Anton (68) has found that the distribution of a drug between tissues and plasma can be altered by changing the extent to which the drug is bound to plasma proteins. For example, sulfinpyrazone displaces the highly bound sulfonamide, sulfaethylthiadiazole, from plasma albumin; as a result the plasma level of sulfonamide declines, and the tissue levels (brain and muscle) rise. Certain other compounds were found to have a similar effect on the distribution of the sulfonamide, and the magnitude of the effect was proportional to the extent to which the compound displaced the sulfonamide from plasma albumin. These findings might have important implications in therapeutics when two or more drugs are administered simultaneously.

Penetration of drugs into erythrocytes and other cells.—Schanker & Nafpliotis (69) have shown that the rates at which a number of drugs enter human red cells are roughly related to the lipid/water partition co-

efficient of the compounds at pH 7.4. Weak organic bases with high lipid solubilities, like antipyrine and aniline, entered the cells very rapidly; those with low lipid solubilities, like serotonin, epinephrine, and norepinephrine penetrated more slowly. Quaternary ammonium compounds, which have very low lipid solubilities, entered at either very slow rates or not at all. A relation between the lipid/water partition ratio and rate of cell penetration was also observed for acidic compounds; however strong acids appeared to enter much more rapidly than strong bases of similar lipid solubility. It was suggested that the entry of organic anions resembles that of the inorganic anions, which also penetrate the red cell at rates greatly exceeding those of cations.

Robbins (70) has reported that the dye 2,8-diaminoacridine (proflavin) penetrates human conjunctiva cells in tissue culture at rates related to the pH of the extracellular fluid. The results were consistent with the view that it is the nonionized form of the dye which penetrates the cell membrane.

Localization of Drugs in Tissue Cells

Accumulation of drugs in body fat.—It has been known for some time that certain drugs of high lipid solubility localize to a considerable degree in body fat [Brodie & Hogben (41); Richards & Taylor (71)]. The mechanism of the distribution appears to be a simple partitioning of lipid-soluble, nonionized molecules between intracellular lipids and body water (41). This type of distribution has been observed for a number of thiobarbiturates, N-alkyl thiobarbiturates, dibenamine, and dibenzyline. The role of body fat in regulating the duration of pharmacologic action of drugs has been discussed frequently (41, 71 to 74).

Intracellular binding of drugs.—Once a drug has penetrated the boundaries of the various tissue cells, its degree of binding to the cell components becomes the most significant factor in determining its distribution in the body. Despite the great importance of intracellular binding, almost nothing is known about it.

The binding of several barbiturates to homogenates of various tissues [Goldbaum & Smith (75)] suggests that the tissue binding and the plasma protein binding of these compounds may be governed by the same principles. On the other hand, the almost limitless capacity of liver-cell nuclei to bind mepacrine [Brodie & Hogben (41); Tomkins & Brodie (76)] suggests a type of binding different from the various proposed types of plasma protein binding.

Tissue localization of endogenous substrates.—Although endogenous substances like serotonin, the catechol amines, amino acids, purines, pyrimidines, various hormones, and so forth are of considerable interest to the pharmacologist, it is beyond the scope of this review to discuss the numerous studies of their physiological distribution. Whenever one of these natural substrates is found to be highly localized in a particular tissue, it may be presumed that the mechanism involved is either active transport or

some type of binding to cell components, or both. For example, serotonin appears to be localized in blood platelets by an active transport mechanism (77 to 80), whereas histamine appears to be concentrated in mast cells because of intracellular binding (81).

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METABOLIC FATE OF DRUGS[1]

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This review is based on the literature from 1957 to May, 1960. For earlier work the reader is referred to the systematic treatment of the metabolism of organic compounds by Williams (1). Discussions on metabolic fate have appeared periodically in the *Annual Review of Biochemistry* (2, 3), and in a sense, this review may be regarded as a continuation of that series. However, somewhat different aspects of the field may be expected to be emphasized in a publication designed primarily for pharmacologists. The pharmacologist is more interested in compounds possessing physiological activity and in results obtained in man. He is also more concerned with a complete accounting of the products of metabolism, because he recognizes that therapeutic or toxic actions may not be caused by the drug itself but by a metabolite which represents only a tiny fraction of the drug. At the present time, few, if any, drugs can be completely accounted for after therapeutic doses in man. However, with the continued introduction of more sensitive and specific analytical methods, it would appear that this goal can ultimately be achieved with dividends by providing more secure knowledge upon which a science of structure-activity relationships can be founded.

The papers which were selected for this review are those concerned with the identification and determination of the excretory products of useful drugs. No reference is made to a large number of studies which did not go further than to describe unidentified spots on paper chromatograms. Because they may give results which are grossly misleading for the intact animal, purely *in vitro* investigations of drug metabolism have not been included. For example, cytochrome oxidase causes the disappearance of catechol amines *in vitro* more rapidly than any known enzyme, but *in vivo* this reaction does not appear to occur (4). The enzymatic aspects of drug metabolism are discussed in other reviews (2, 4, 5, 6).

Hypnotics

Barbiturates.—The study of the fate of this series of drugs continues to generate many publications, particularly by German and Japanese investigators, but, except for the work described below, the only new metabolites characterized are some oxidative and hydrolytic products of drugs containing the 1-cyclohexenyl group (7, 8). Other reviews (9 to 12) summarize the evidence that the barbiturates are metabolized by four different routes: oxidation of substituents in the 5-position of the barbituric acid ring,

[1] The following abbreviation has been used: TEPA (triethylenephosphoramide).

hydrolysis (generally a minor reaction), oxidation of alkyl groups attached to a nitrogen atom, and desulfuration of thiobarbiturates. Although it is clear that part of the sulfur of thiobarbiturates is converted to inorganic and organic sulfate (13), evidence for the direct conversion of thiobarbiturates to ordinary barbiturates *in vivo* has not been above objection, inasmuch as the sulfur of thiobarbiturates can be oxidized by the peroxide in ethyl ether employed in extraction. Recently the excretion of barbital after the administration of thiobarbital in man has been demonstrated with a more satisfactory method (14). Reports (15, 16) that N-methylation of a barbiturate (nor-evipal) occurs to some extent in the mouse are unconvincing.

Some new experiments (17) on 2-C^{14}-labeled Phanodorn (5-Δ^1-cyclohexenyl, 5-ethylbarbituric acid) in the rat have indicated that the metabolites can be accounted for as follows: 0 to 1.5 per cent unchanged drug, 12 to 13 per cent 3-hydroxycyclohexenyl derivative, 73 to 74 per cent 3-oxo-

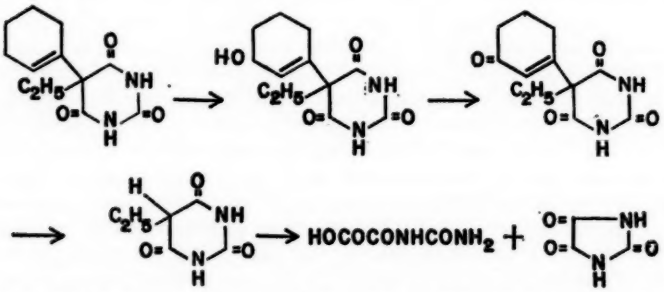


FIG. 1. Metabolism of Phanodorn (5-Δ^1-cyclohexenyl, 5-ethyl barbituic acid).

cyclohexenyl derivative, 9 per cent ethylbarbituric acid, 1 to 2.5 per cent oxaluric acid, 1 to 2 per cent parabanic acid (see Fig. 1). A small amount of ethylbarbituric acid has also been found after the administration of barbital and 5-ethyl, 5-vinylbarbituric acid (18). The removal of substituents in the 5-position probably involves unstable intermediates with a hydroxyl group on the carbon atom immediately adjacent to the barbituric acid ring. The mechanism of the formation of oxaluric and parabanic acid from ethylbarbituric acid is not at all clear. One possible route would be hydrolysis and decarboxylation to yield butyryl urea, which could be converted to oxaluric acid through ω-oxidation followed by β-oxidation.

The formation and subsequent degradation of monoalykl derivates may explain the failure of all other workers to account fully for the products of barbiturate metabolism. This interesting work requires confirmation.

Methyprylon.—This drug (3,3-diethyl-5-methylpiperidine-2,4-dione) appears to be metabolized primarily through a reaction which may be formulated as a dehydrogenation but possibly results from hydroxylation fol-

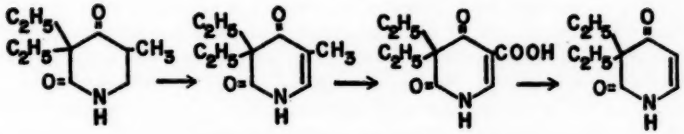


FIG. 2. Metabolism of methyprylon.

lowed by dehydration. As illustrated in Figure 2, the oxidation product (3,3-diethyl-2,4-dioxo-5-methyltetrahydropyridine) has been isolated from the urine of man, the rat, and the dog (19, 20, 21). A similar dehydrogenation was described earlier for 3,3-diethylpiperidine-2,4-dione (22). In the dog the methyl group of the dehydrogenation product of methyprylon has been shown to be oxidized to a hydroxymethyl derivative and then to a carboxylic acid; both of these compounds are excreted in the urine as glucuronides. Part of the carboxylic acid derivative is decarboxylated to 3,3-diethyl-2,4-dioxotetrahydropyridine which also appears in the urine (21).

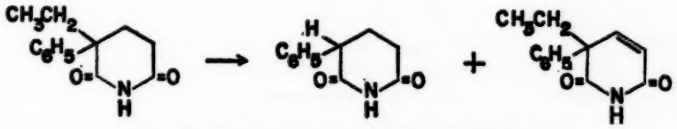


FIG. 3. Unconjugated metabolites of glutethimide.

Glutethimide.—After the administration of this drug to dogs or rats, only traces of unchanged α-ethyl, α-phenylglutarimide appear in the urine (19, 23). In the dog most of the products of the drug are excreted conjugated with glucuronic acid. The unconjugated metabolites, which account for less than 10 per cent of the dose, consist of the dealkylation product, α-phenylglutarimide, and the dehydrogenation product, α-ethyl, α-phenylgluconimide (24 to 27) (Fig. 3). Three glucuronides have been isolated as crystalline derivatives after treatment with diazomethane and acetic anhydride. The aglycones have been shown to have the structures illustrated in Figure 4 (27).

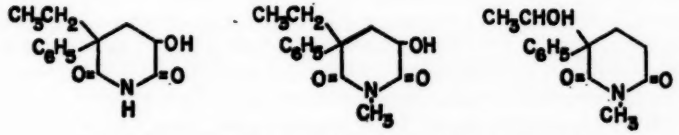


FIG. 4. Aglycones of glucuronides formed from glutethimide.

These compounds prove that hydroxylation occurs in both the ethyl group and the glutarimide ring. The isolation of a 1-hydroxyethyl derivative is noteworthy inasmuch as such compounds have been proposed as inter-

mediates in dealkylations involving rupture of a carbon-carbon bond. The N-methyl group in two of the compounds was probably introduced by the treatment with diazomethane. Although N-methylation has long been recognized as an important biochemical reaction of natural substrates and has been observed in connection with the metabolism of a few foreign amines (e.g., pyridine, quinoline, *p*-methylaminoazobenzene), there is no satisfactory evidence for methylation of an imido group.

Ethinamate and hydroxydione.—The metabolism of ethinamate (1-ethynylcyclohexyl carbamate) (Fig. 5) has been studied in rats. Hydrolytic cleavage of the ester is a minor reaction. Most of the drug is hydroxylated, but the position of the hydroxyl group in the cyclohexyl ring has not been established. About half of the hydroxyethinamate is excreted as such; the remainder is excreted as a glucuronide (28).

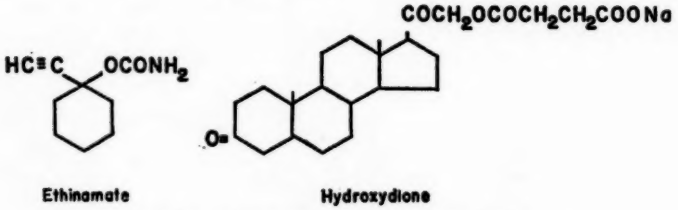


FIG. 5. Structures of ethinamate and hydroxydione.

Hydroxydione (pregnane-21-ol-3,20-dione hemisuccinate sodium (Fig. 5), which has been used for intravenous and basal anesthesia, is metabolized in man by reduction of the 3-keto group to a hydroxyl group which is then conjugated with glucuronic acid. The ester linkage is hydrolyzed. Among the metabolites identified, the principal excretory product is the monoglucuronide of 21,3-α-pregnanediol-20-one; however, the 21-hydroxy group is also partly conjugated. Inasmuch as only 15 to 20 per cent of the drug can be accounted for, oxidation of the side chain to form derivatives of 3-hydroxy- and 3-keto-etiocholanic acid has been suggested (29 to 32).

ANTIEPILEPTICS

Drugs containing an N-alkyl group.—Convincing evidence has been presented that the five drugs shown in Figure 6 (N-methyl derivatives of barbituric acid, hydantoin or oxazolidine 2,4-dione) are almost completely demethylated in man (33). When these drugs are administered chronically in the treatment of epilepsy the concentrations in the body of the products of demethylation far exceed those of unchanged drug. The demethylated derivatives of the five drugs are known to possess antiepileptic activity. Therefore, there is a serious question whether any therapeutic benefits are achieved by administering any of these drugs in preference to the products of demethylation. This is a problem which can be solved only by means of controlled clinical investigation.

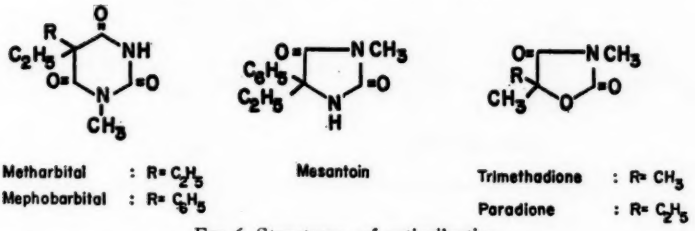


FIG. 6. Structures of antiepileptics.

Barbital and 5,5-dimethyloxazolidine-2,4-dione, the products of demethylation of metharbital and trimethadione, respectively, are eliminated almost completely unchanged, although excretion in man has not been studied as thoroughly as in experimental animals (9, 34). It is not known whether 5-ethyl,5-methyloxazolidine-2,4-dione resulting from the demethylation of paradione completely resists chemical alteration in the body; at least a portion of the compound is excreted unchanged (35). Phenobarbital, which is formed from mephobarbital, is excreted partly unchanged and partly as *p*-hydroxyphenobarbital (36 to 39), which for all practical purposes lacks hypnotic activity (39). Presumably, phenobarbital is also metabolized to other products which are still unknown. In the dog, *p*-hydroxyphenobarbital is excreted entirely conjugated, for the most part with glucuronic acid (39), but in man it is only partly conjugated, and the nature of the conjugation is uncertain (37, 38, 39). Nirvanol (5-ethyl,5-phenylhydantoin) produced by the demethylation of mesantoin is also excreted by man partly unchanged and partly as *p*-hydroxynirvanol (40, 41). The *p*-hydroxynirvanol in dog urine is largely conjugated with glucuronic acid (40); it is not known whether this is also true in man.

Diphenylhydantoin and primidone.—A study with N^{15}-labeled diphenylhydantoin (Fig. 7) has proved that hydrolysis of the hydantoin ring does not occur to an important extent in dogs (42). In man and the dog, a large part of the drug is converted to 5-(*p*-hydroxyphenyl),5-phenylhydantoin, which is subjected to further metabolic alteration (43). Both species excrete about 50 per cent of a dose of diphenylhydantoin as the *p*-hydroxy derivative; 75 per cent of this is conjugated with glucuronic acid, and less than 1 per cent is excreted in the free form (42, 43). Hydroxylation of a phenyl group causes the carbon atom in the 5-position of the hydantoin ring to become asymmetric. The phenolic metabolite in the urine of man consists almost entirely of the levorotatory isomer. The dog excretes both isomers with a slight preponderance of the dextrorotatory form (43).

Primidone (5-ethyl,5-phenyl-4,6-dioxo-hexahydropyrimidine) (Fig. 7) is metabolized in part through oxidation to phenobarbital (44). In man the extent of this conversion is of the order of 15 per cent; in dogs it amounts to only 5 per cent. In rats ethylphenylmalonamide may also be a metabolite (45).

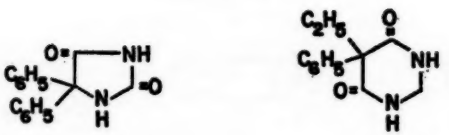


FIG. 7. Structures of diphenylhydantoin and primidone.

ANALGESICS

Morphine and related drugs.—The literature on the narcotic analgesics up to 1957 has been reviewed (46). More recent papers on morphine (47 to 51), levorphanol (52), heroin (53), and codeine (54) are generally in agreement with earlier reports and do not require discussion. A study on the narcotic antagonist, levallorphan (levo-3-hydroxy-N-allylmorphinan), has revealed that all of the common laboratory animals excrete small amounts of the free and conjugated deallylation product, 3-hydroxymorphinan, in the urine (55). Another metabolite which differs from levallorphan in possessing an extra oxygen atom has been isolated from rat urine but not yet identified. Rats excrete only 5 per cent of a large dose of levallorphan as unchanged drug in its free and conjugated forms (55). In contrast, 44 to 60 per cent of a dose of nalorphine can be accounted for as free and conjugated drug in dogs (56). Studies with liver microsomes have shown that nalorphine can be deallylated to normorphine or conjugated with glucuronic acid (48, 57).

6-Dimethylamino-4,4-diphenyl-3-hexanone, which has received clinical use in Japan, has been reported to be metabolized in rats through reduction of the ketone group to a hydroxyl group (58). Whether a similar reduction occurs with methadone, 6-dimethylamino-4,4-diphenyl-3-heptanone, is not known.

Anileridine and propoxyphene.—Anileridine (Fig. 8), which differs from meperidine in having a *p*-aminophenylethyl in place of a methyl group, is metabolized by hydrolysis of the ester and acetylation of the amino group. In man four metabolites have been determined in the urine: unchanged drug (5 per cent), anileridine acid (7 to 14 per cent), acetyl anileridine (0.5 to 2 per cent), and acetyl anileridine acid (1 to 2 per cent). In man, the rat, and the guinea pig an additional 15 to 35 per cent of the dose is excreted as unidentified diazotizable substances; in the rat this fraction consists largely of *p*-acetylaminophenylacetic acid or some closely related compound (59).

The fate of α-dl-propoxyphene (Fig. 8), has been studied in rats and man. Rats given the drug labeled with C^{14} in one of the N-methyl groups excrete 38 per cent of the radioactivity in the expired air, 35 per cent in the feces, and 15 per cent in the urine. The product of mono-demethylation has been isolated from human urine by means of a dinitrophenyl derivative (60).

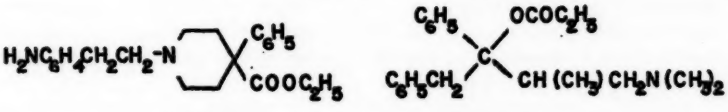


FIG. 8. Structures of anileridine and propoxyphene.

TRANQUILIZING AGENTS

Phenothiazine derivatives.—Promazine and chlorpromazine (Fig. 9) appear to be metabolized by sulfoxidation, hydroxylation, and demethylation. Chlorpromazine sulfoxide has been isolated from the urine of dogs; in man it accounts for about 5 per cent of a dose of the drug (61). The

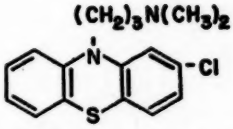


FIG. 9. Structure of chlorpromazine.

pharmacological actions of this metabolite differ qualitatively and quantitatively from chlorpromazine (62); it is a weak sedative in man and is thought to contribute little to the action of the parent compound (63). Sulfoxide metabolites of promazine (64) and mepazine (10[(1-methyl-3-piperidyl)methyl] phenothiazine)(65) have also been reported. A study of the fate in rats of chlorpromazine labeled with C^{14} in one of the methyl groups has proved demethylation to be an important process; as much as 17 per cent of the isotope is exhaled as carbon dioxide in six hours, and considerable amounts are eliminated during the following six hours (66). After the administration of S^{35}-labeled promazine to dogs, the products of monodemethylation of the drug and the corresponding sulfoxide are excreted in the urine, but these two metabolites together account for less than 5 per cent of the dose (64). In man both methyl groups may be removed from the dimethylamino group of chlorpromazine; the mono-methyl and the completely demethylated sulfoxide derivatives have been stated to be the major extractable metabolites, but quantitative data are lacking (67). Removal of the entire dimethylaminopropyl group apparently does not occur, at least in dogs; this is suggested by the fact that radioactive sulfate is a product after S^{35}-labeled phenothiazine but not after labeled promazine (64). However, sulfate has been reported as a metabolite of chlorpromazine in mice (68). Treatment of human urine with β-glucuronidase has been shown to liberate additional metabolites of chlorpromazine that have not yet been definitely identified but may be presumed to contain hydroxyl groups in the

phenothiazine ring (69, 70). No metabolites of promazine or chlorpromazine containing the sulfone group have been detected; chlorpromazine sulfone has been shown to be rapidly metabolized to products of unknown structure by liver microsomes (71).

Reserpine.—In all species examined, only minute amounts of reserpine (Fig. 10) are excreted unchanged in the urine. The earliest study of the fate of this drug revealed that 30 to 60 per cent of an oral or intravenous dose was excreted by mice as 3,4,5-trimethoxybenzoic acid, a compound which is excreted entirely unchanged by this species (72). However, it seems unlikely that hydrolysis to methyl reserpate and trimethoxybenzoic acid is the primary biochemical transformation of reserpine. Rats given reserpine by mouth excrete 22 per cent of the drug in the urine and 45 per cent in the feces as methyl reserpate. However, if the same dose is

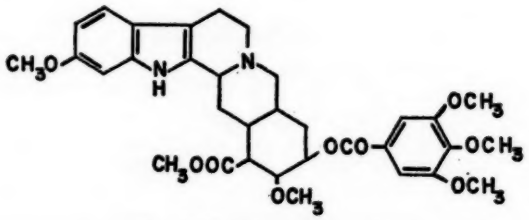


FIG. 10. Structure of reserpine.

given subcutaneously or intraperitoneally, the urinary excretion of methyl reserpate amounts to less than 1 per cent of the dose. It has been demonstrated that the intestine of the rat differs from that of the dog or the monkey in being capable of hydrolyzing reserpine to methyl reserpate. A considerable part of the methyl reserpate formed in the intestine of the rat appears to be absorbed and excreted unchanged in the urine. The very small amount of this compound in the urine after parenterally alministered reserpine indicates that methyl reserpate is not an important intermediary product in the rat when the oral route is avoided. In the dog and the monkey only small amounts of methyl reserpate are found in the tissues and excreta, regardless of the route of administration (73).

Apart from hydrolysis, the only chemical change which has been demonstrated for reserpine is O-demethylation. Rats injected with drug labeled with C^{14} in the 4-methyl group of the trimethoxybenzoyl moiety excrete 24 per cent of the radioactivity in the breath in six hours (74). O-Demethylation products of reserpine and trimethoxybenzoic acid (syringoyl methyl reserpate and syringic acid) have been detected in rat tissues (75), but adequate quantitative data to assess the importance of these metabolites is lacking. No correlation has yet been found between the concentrations of reserpine or its metabolites in the brain and the duration of pharmacological effects (75, 76, 77). It may be that reserpine or one of its metabolites causes

some change in body chemistry which persists long after the drug and its products are completely eliminated, but this can scarcely be accepted as proved until the distribution and fate of all of the metabolites of reserpine are known.

Meprobamate.—The administration of C^{14}-labeled meprobamate to man, dogs, or rats leads to the urinary excretion of four radioactive products in addition to unchanged drug (Fig. 11) (10 to 20 per cent of the dose) (78, 79). Two metabolites appear to be alcoholic derivatives obtained from oxidation of the methyl and propyl groups. One of these has been proved to be 2-hydroxymethyl,2-*n*-propyl-propane-1-3,diol dicarbamate; this compound, which lacks sedative activity, accounts for 60 per cent of the urinary metabolites in dogs (78). The other two products are glucuronic acid conjugates. Acid hydrolysis of one of these yields the hydroxymethyl derivative of meprobamate; the combination with glucuronic acid may involve either the hydroxyl group or the amino groups of the metabolite. Treatment of the other conjugate with acid yields unchanged meprobamate; this substance appears to be an N-glucuronide (80).

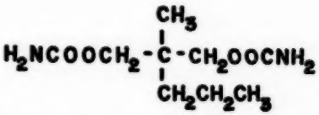


FIG. 11. Structure of meprobamate.

N-glucuronides have been considered as metabolites of aromatic amines for over 10 years (81, 82, 83), but much remains to be learned about this class of compounds. They are very rapidly hydrolyzed in acidic solutions but are not attacked by β-glucuronidase (84). Their formation from uridine diphosphate glucuronic acid is catalyzed by the microsomal enzyme system involved in the formation of ether and ester glucuronides (84); however, they are also formed in aqueous solution by the nonenzymatic condensation of amines with glucuronic acid (81, 85, 86). Other drugs reported to form N-glucuronides are 4,4-diaminodiphenylsulfone (86), sulfanilamide (87), and sulfapyridine (88).

CENTRAL NERVOUS SYSTEM STIMULANTS

Iproniazid.—This monoamine oxidase inhibitor, which has been employed clinically as a psychomotor stimulant, is subjected to two different chemical reactions in mice, rats, and rabbits (89) (Fig. 12). One part of

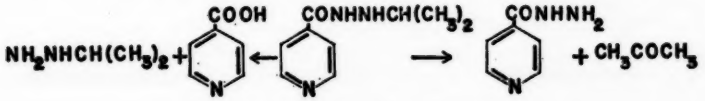
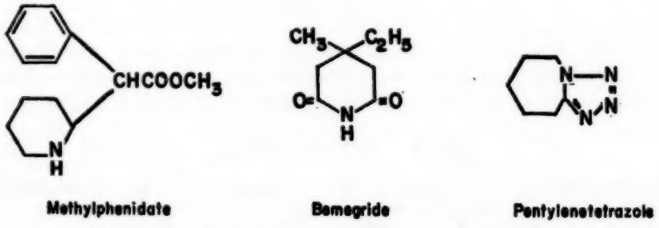


FIG. 12. Metabolism of iproniazid.

the drug is hydrolyzed to isonicotinic acid and isopropylhydrazine; another part is dealkylated to form isoniazid (isonicotinic acid hydrazide) and acetone. After the administration to rats of iproniazid labeled with C^{14} in the isonicotinic acid moiety, 98 per cent of the radioactivity appears in the urine in 24 hours, and almost all of this is in the form of isonicotinic acid and unchanged drug (90). These findings suggest that the principal metabolic pathway for iproniazid involves hydrolysis to isonicotinic acid and isopropylhydrazine, because, if isoniazid were formed to any considerable extent, one would expect to find N-acetyl,N′-isonicotinoylhydrazine and the isonicotinoylhydrazones of pyruvic and α-ketoglutaric acids in the urine (91, 92). However, other investigators have found the acetyl derivative of isoniazid in urine after the administration of iproniazid, although quantitative data are not available (89). When iproniazid containing C^{14} in the isopropyl group is administered to mice, rats, or rabbits, as much as 60 per cent of the isotope is excreted in the breath. Radioactive acetone can be detected in the tissues and excreta; very large doses of iproniazid appear to inhibit the oxidation of acetone to CO_2. The labeled acetone could arise from the dealkylation of either iproniazid or isopropylhydrazine. Two metabolites of iproniazid detected in the urine by paper chromatography have been considered to be isopropylhydrazones of normal tissue constituents, but they have not been definitely identified (89).

Although isopropylhydrazine is known to be quite potent with respect to both toxicity and mono, amine oxidase inhibition, the role of this compound in causing the effects of iproniazide *in vivo* has yet to be clarified. In this connection it is interesting to note that benzohydrazide ($C_6H_5CONHNH_2$) and *p*-chloro- and *p*-methyl-benzohydrazides, which are quite toxic, are extensively hydrolyzed in rabbits to the corresponding carboxylic acids and hydrazine. The less toxic salicylohydrazide and *p*-hydroxybenzohydrazide are less extensively hydrolyzed, and are metabolized primarily through conjugation with glucuronic acid (93, 94, 95).

Other stimulants.—Methylphenidate (Fig. 13) is metabolized primarily through hydrolysis of its ester linkage. After the administration of drug labeled with C^{14} in the carbonyl group to rats, none of the isotope appears in the breath, and only minute amounts are found in the feces. A negligible



Methylphenidate Bemegride Pentylenetetrazole

FIG. 13. Structures of central nervous system stimulants.

quantity of the drug is excreted unchanged. The major urinary product is phenyl(2-piperidyl) acetic acid (62 per cent of the dose). Two other metabolites have been detected but their structures are unknown (96).

The fate of bemegride (Fig. 13) has been studied in a patient who received this drug for the treatment of poisoning with phenobarbital. Unchanged bemegride and a metabolite containing a hydroxyl group were isolated in pure form from the urine. The metabolite gives a positive iodoform test; this reaction proves conclusively that the hydroxyl group is located in the ethyl group on the carbon atom immediately adjacent to the glutarimide ring (97).

Some new work on the metabolism in the rat of pentylenetetrazole (Fig. 13) labeled with C^{14} has yielded interesting results (98). Most of the injected radioactivity appears in the urine in 48 hours. When the urine or extracts of it are subjected to paper chromatography, only one compound can be detected, and this is indistinguishable from unchanged drug. On the other hand, by means of biological methods it has been shown quite clearly that the drug is metabolized to an inactive compound. Furthermore, partial hepatectomy prolongs the sojourn of the drug in the body and nephrectomy is without effect. It would seem that the only explanation for these apparently contradictory observations is that pentylenetetrazole is metabolized in the rat to a very labile conjugate of unknown structure which reverts to the drug in urine. However, it is difficult to imagine what kind of conjugation could be involved.

Cardiac Glycosides

Recent research has revealed that hydroxylation and cleavage of the sugar moieties are important reactions in the metabolism of the cardiac glycosides. In man and the rat digitoxin yields several metabolites, one of which is digoxin (12-hydroxydigitoxin) (99). In the rat injected with digitoxin the digitoxose chain undergoes a stepwise degradation to yield the bis-digitoxosides and the mono-digitoxosides of digitoxigenin and digoxigenin. The principal metabolite of digitoxin in the rat is digoxigenin-bis-digitoxoside (62 per cent of the dose). Unchanged drug accounts for 6 per cent of the dose and digoxin, 20 per cent (100, 101).

The cleavage of the sugars of cardiac glycosides has also been demonstrated in a study of K-strophanthoside and thevetin (102). After oral or subcutaneous administration of strophanthoside, unchanged drug can be detected in the bile but not in the excreta. However, both the urine and the feces contain the product (cymarin) formed from the cleavage of two molecules of glucose. Homogenates of tissues do not cause hydrolysis of strophanthoside but incubation with rat feces leads to a rapid formation of cymarin. Similar findings have been obtained with thevetin. This work raises the question of the relative importance of tissue enzymes and the intestinal flora in the chemical transformations observed with other cardiac

glycosides. The literature on this problem is quite unsatisfactory. For example, some workers have failed to detect any oxidation of digitoxin *in vitro* (103), whereas others find that oxidation and cleavage occur not only in the liver but in blood or plasma (104). One is forced to consider the possibility that the presence or absence of bacteria may play some part in causing the discrepancy in results.

The excretion of the cardiac glycosides into the bile appears to depend upon their polarity or their partition coefficients. After parenteral administration to rats, more than 60 per cent of a dose of a polar drug like ouabain (105) or lanatoside C (106) appears unchanged in the bile. Under the same conditions, less than 10 per cent of a dose of a less polar drug like digitoxin or digoxin enters the bile (106). Secretion into the bile and a limited reabsorption from the gastrointestinal tract provide an explanation for the short duration of action of ouabain and the lanatosides, but further work will be necessary to establish this point.

Sulfonamides

Carbonic anhydrase inhibitors.—A study of the metabolic fate of 2-benzothiazolesulfonamide has provided an explanation for its failure to inhibit

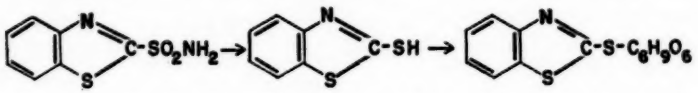
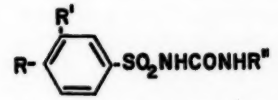


Fig. 14. Metabolism of 2-benzothiazolesulfonamide.

carbonic anhydrase *in vivo* (107). After intravenous administration to dogs, no unchanged drug appears in the urine, but a metabolite accounting for about 25 per cent of the dose can be isolated. This compound has been proved to be the glucuronide of 2-mercaptobenzothiazole (Fig. 14). As such it is the first thioglucuronide (S-glucuronide) to be isolated in pure form. Small amounts of 2-mercaptobenzothiazole and 2-hydroxybenzothiazole have also been isolated from urine. The reduction of the sulfonamide group to a sulfhydryl group is a metabolic reaction which has not been described previously. In the light of earlier observations that thiophenol leads to an increased excretion of ethereal sulfates as well as glucuronides (1), it is possible that mercaptobenzothiazole is also conjugated with sulfuric acid. Acetazoleamide, a carbonic anhydrase inhibitor active *in vivo,* is excreted largely (more than 70 per cent) unchanged (108).

Sulfonylureas.—These drugs, which are useful in the treatment of diabetes mellitus, are metabolized by reactions of substituents in the benzene ring and by modification of the substituted urea group (Fig. 15). In man the only important reaction of tolbutamide is oxidation of the *p*-methyl group to a carboxylic acid group; practically all of the drug is excreted as N-4-carboxybenzenesulfonyl,N′-*n*-butyl urea, which has no hypoglycemic activity (109, 110). Dogs convert tolbutamide to *p*-toluenesulfonyl urea and *p*-toluenesulfonamide, but quantitative data are lacking (111, 112). In the



	R	R'	R''
Tolbutamide :	CH_3	H	n- butyl
Chlorpropamide :	Cl	H	n- propyl
Metahexamide :	CH_3	NH_2	cyclohexyl
Carbutamide :	NH_2	H	n- butyl

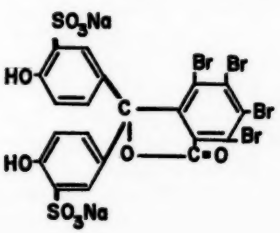
FIG. 15. Structures of the sulfonylureas.

dog chlorpropamide is excreted partly unchanged (27 to 33 per cent), and partly as *p*-chlorobenzenesulfonyl urea (35 to 40 per cent) and the corresponding sulfonamide (16 to 24 per cent) (113). The drug appears to undergo less change in man (114), but *p*-chlorobenzenesulfonamide is also a metabolite in this species (113). Metahexamide has not been studied in man, but the dog excretes some of this drug as 3-amino-4-methylbenzenesulfonamide (115). The only data on the fate of carbutamide were obtained by use of the Bratton-Marshall method for aromatic amines; the results indicated that in man about one third of the excreted amino group was acetylated (116). Now that it is known that aromatic amines may be excreted as N-glucuronides and N-sulfates as well as acetylated derivatives and that some of these conjugates are very labile under conditions required for diazotization, this classical method for dividing excreted amines into free and acetylated fractions does not necessarily yield a meaningful result.

DIAGNOSTIC AGENTS

Bromsulfalein.—Employed clinically to test hepatic function, this drug (Fig. 16) is largely metabolized to derivatives which have the same color. After the administration of S^{35}-labeled bromsulfalein to dogs, four metabolites, none colorless, can be detected in the bile (117). The metabolites appear to be similar in man and all other species studied, although marked quantitative differences occur (118, 119, 120). Attempts to demonstrate conjugation with sulfuric or glucuronic acid have been unsuccessful (121, 122,

FIG. 16. Structure of bromsulfalein.



123), although the drug apparently forms a glucuronide in the presence of liver microsomes (124). A number of studies have shown that the metabolites contain amino acids (120, 122, 123, 125). Although none of the metabolites has been definitely identified, it sems likely that conjugation with glutathione is involved. Hydrolysis of the metabolites yields cysteine, glycine, and glutamic acid (120, 122, 125). Furthermore, in two of the metabolites the sulfur of the cysteine has been demonstrated to be involved in a thioether linkage (125). Compounds similar to the metabolites are formed by the reaction of the drug with glutathione or cysteine *in vitro* (125, 126, 127). The reaction of bromsulfalein with a sulfhydryl group *in vivo* could involve replacement of either a bromine or a hydrogen atom in one of the aromatic rings. Although loss of a bromine atom would seem more likely, an analysis of one of the metabolites isolated from rat bile after the administration of labeled drug indicated that the ratio of Br to S^{35} was the same as in the drug itself (118). It has been shown that after administration to rats one of the metabolites is converted in part to a second metabolite; when the second metabolite is administered, it appears in the bile entirely unchanged (128). If the metabolism of bromsulfalein involves a condensation with glutathione, some of the products detected may arise by stepwise removal of glycine and glutamic acid and the acetylation of the cysteine residue to form a mercapturic acid. The possibility of the introduction of two sulfhydryl-containing moieties into bromsulfalein seems less likely.

Evidence that a condensation with glutathione is the primary step in the formation of mercapturic acids has been strengthened in the past few years. However, larger SH-containing peptides and proteins may sometimes be involved (129). Several foreign compounds leading to mercapturic acids have been shown to cause a decrease in liver glutathione (130). The incubation of rat liver slices with naphthalene leads to the formation of a compound that is almost certainly S-(1,2,dihydro-2-hydroxy-1-naphthyl)-glutathione (131). The ingestion of 1-bromobutane causes the excretion of three metabolites containing sulfur. One of these, after hydrolysis, yields glutamic acid, glycine, and S-butyl-L-cysteine; the second metabolite yields only glycine and S-butyl-L-cysteine. The third compound is *n*-butylmercapturic acid (N-acetyl-S-butyl-L-cysteine) (132).

Anticancer Agents

The availability of a recent review (133) on the fate of drugs employed in the treatment of cancer makes it permissible to confine the present discussion to busulfan and some of the phosphoramides.

Busulfan.—This drug has been studied after separate labeling of the sulfur and the carbon of the tetramethylene chain. After the administration of the S^{35}-labeled drug, the isotope appears in the urine largely in the form of methanesulfonic acid (134, 135). The administration of the C^{14}-labeled drug to man or the rat causes the appearance of a large number of radioactive compounds in the urine (134, 136); this result has been interpreted

as suggesting alkylation of unknown body constituents. In the rat and the rabbit the major metabolite is 3-hydroxytetrahydrothiophene-1,1-dioxide (137, 138) (Fig. 17). The pathway for the formation of this compound may involve the alkylation of a cysteine-containing substance by the drug to form the S-β-alanyltetrahydrothiophenium ion which is then converted to tetrahydrothiophene, which is metabolized by oxidation to the sulfone and hydroxylation.

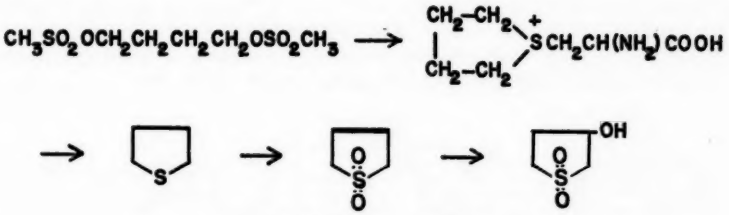


FIG. 17. Metabolism of busulfan.

Phosphoramides.—Inasmuch as alkylating agents have been thought to be highly reactive under biological conditions, it was surprising to discover that considerable quantities of unchanged busulfan appear in the urine of rats (134, 135). Another alkylating agent, N,N′,N″-triethylenephosphoramide (TEPA) is even more stable *in vivo*. In rats this drug is excreted largely unchanged (139). However, in man and the mouse most of it is converted to inorganic phosphate (136, 140). In the rat, the dog, and the rabbit TEPA is a major metabolite of thio-TEPA (Fig. 18) (141, 142); the

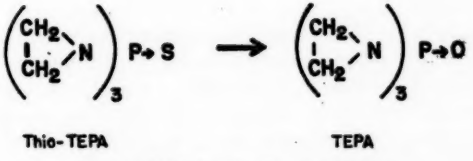


FIG. 18. Metabolism of thio-TEPA.

mouse also degrades this compound almost completely to inorganic phosphate (141). Another thiophosphoramide, OPSPA, which differs from thio-TEPA in having a morpholino group in place of one of the ethylene imino groups, has also been shown to be excreted by man and the rat largely as the oxygen analogue (143). The fact that some of the alkylating agents are excreted unchanged should not be taken to suggest that any therapeutic benefit derived from these substances is not the result of the alkylation of some critical tissue constituent. However, that these drugs do act through alkylation is also unproved.

Miscellaneous Drugs

Nicotine.—Studies in the rat (144) and in the dog (145) with nicotine uniformly labeled with C^{14} have demonstrated that practically all of the isotope is eliminated in the urine. Five metabolites which have been isolated (146 to 149) from dog urine are shown in Figure 19. The position of the hydroxyl group in hydroxycotinine has not been established. All of these compounds with the exception of N-methylnicotine (which has not been sought) have also been detected in the urine of man after smoking or after the ingestion of nicotine (150). The administration to the dog of cotinine, which is less toxic than nicotine, causes the excretion of hydroxycotinine and desmethylcotinine plus some N-methylcotinine (148, 149); presumably,

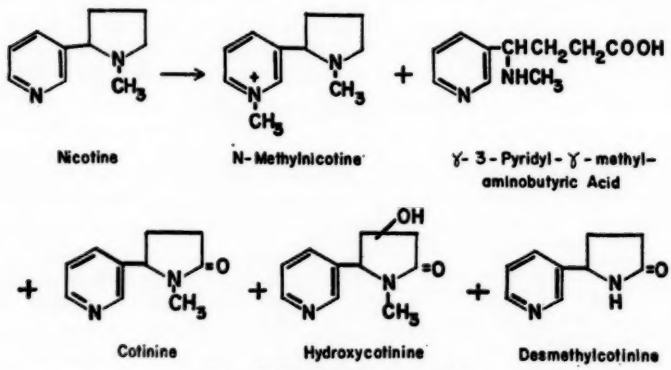


Fig. 19. Metabolism of nicotine.

the latter compound is also a metabolite of nicotine. Earlier work indicated that cotinine was formed primarily by the cyclization of γ-3-pyridyl-γ-methylaminobutyric acid; this reaction proceeds readily *in vitro* under physiological conditions (146, 147). However, the oxidation of nicotine to cotinine by liver microsomes does not appear to involve the amino acid to an important extent; 5′-hydroxynicotine seems a more likely intermediate (151, 152). Also, cotinine could not be detected in the urine of rabbits given γ-3-pyridyl-γ-methylaminobutyric acid (151). 6-Hydroxynicotine is a product of the metabolism of nicotine by bacteria (153, 154); whether this compound is also formed in mammals is not known.

Thiouracil.—The fate of this drug is of interest because it illustrates S-methylation as a biochemical reaction of foreign compounds. After the administration of S^{35}-labeled thiouracil to rats, 60 per cent of the isotope is excreted in the urine as unchanged drug, 6 per cent as 2-methylthiouracil, and 5 per cent as inorganic sulfate (Fig. 20) (155). The desulfuration product, uracil, has also been isolated (156). Other drugs shown to undergo S-methylation are 5-iodo-2-thiouracil (157) and 6-mercaptopurine (158).

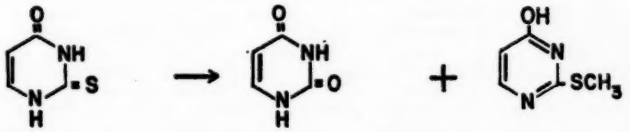


FIG. 20. Metabolism of thiouracil.

Other drugs.—Because of limiations in space it is not possible to discuss recent work on atropine (159), 2-(butylaminomethyl) 1,4-benzodioxane (160), carbromal (161), chlorzoxazone (162), N,N-diethyltryptamine (163), N,N-dimethyltryptamine (164), Dimedion (165), ethyl trichloramate (166), hydralazine (167), isocarboxazid (168), lysergic acid diethylamide (169), mescaline (170), papaverine (171), and zoxazolamine (172).

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EFFECTS OF TEMPERATURE ON THE ACTION OF DRUGS[1,2]

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The use of hypothermia in medicine and surgery emphasizes the need for fundamental data on the action of drugs at different temperatures, but of primary importance are the effects of temperature on biochemical reactions in general. Studies of the influence of temperature on biological reactions are useful in the separation of physical and chemical processes, the latter being highly sensitive to temperature change, but they are not always easy to interpret. Calculation of a temperature coefficient is helpful in interpretation. One of the simplest and most commonly used, Q_{10}, varies with temperature except for the rare cases in which rate is an exponential function of temperature. Usually it decreases with increase in temperature and, at low temperatures, may greatly exceed the range of 2 to 3 which van't Hoff found for simple chemical reactions (41). If the logarithm of the rate of a reaction is plotted as a function of the reciprocal of the absolute temperature, a straight line is sometimes, but not always, obtained. A straight line is to be expected from the Arrhenius equation, in which the slope of the line, μ or E, has theoretical meaning (52). Where straight lines do not result (this in itself is informative), other mathematical treatments can be used. One of the most useful, the "b, α rule," has recently been given theoretical meaning by Bělehrádek (11) and has been shown to describe a wide variety of temperature-dependent processes such as protoplasmic viscosity, permeability, diffusion, and some enzyme reactions. According to Bělehrádek, the rate of biological processes is determined primarily by protoplasmic resistance opposing free movement of molecules. Application of this concept to drug-tissue interactions at different temperatures should certainly be made.

When this subject was last reviewed (31), several hypotheses were proposed to explain the differences in the actions of drugs at different temperatures. Since then the use of hypothermia and local cooling has become widespread, but there have been very few original investigations of fundamental biochemical processes at different temperatures. In contrast, there has been much interest in the practical applications of temperature differences and their effects on the action of drugs. This review does not include effects of hypothermia or cold exposure on secretion of adrenal cortical or medullary hormones. Also, the important topic of prevention of ventricular fibrillation

[1] The literature reviewed includes a few important publications which appeared between 1946 and 1955 and most of those published between 1955 and March, 1960.

[2] Abbreviations used in this chapter include: BAL (2,3-dimercaptopropanol).

during hypothermia is not included, since it has been fully discussed (3, 4, 23, 25, 45).

Environmental Temperature

Many authors still report differences in the toxicity of various drugs at different environmental temperatures. This type of experiment is not informative when the object of the research is an understanding of the fundamental action of a drug, and, sadly, some authors are still surprised to find that environmental temperature affects toxicity by changing body temperature. This has been recognized for many years and is entirely to be expected in small animals. The very careful work of Usinger (106) shows how body temperature of white mice can vary with environmental temperature. He found the body temperature to be 35.8°, 34.0°, 34.7°, and 37.2°C. at environmental temperatures of 20°, 25°, 30°, and 35°C., respectively. It would be preferable to study the effects of body temperature on toxicity, but it is certainly essential to control environmental temperature, and to measure body temperature, when studies of toxicity are made using small rodents. There is some practical value in experiments that show increased toxicity by increased absorption through the skin at high environmental temperatures (for instance, war gases), but the above precautions are necessary for correct interpretation of results.

Other physiological effects of environmental temperature are important in studies of toxicity. For instance, an environmental temperature of 38°C. is lethal to mice in three hours at a relative humidity of 20 per cent (2). The minimal rate of oxygen consumption in mice is at an environmental temperature of 30°C., but the rate increases both above and below this temperature. Blood flow through skin and subcutaneous tissue varies greatly with environmental temperature. Since small animals are so often used in studies of temperature and the action of drugs, these factors may influence results.

Shemano & Nickerson (95) found that there is a critical environmental temperature above which a given drug produces a rise in body temperature and below which it produces a fall. In rats this critical temperature is 30°C. for Hydergine (a mixture of the methanesulfonates of dihydroergocornine, dihydroergocristine, and dihydroergocryptine), ergotamine, *N, N*-diethyl-D-lysergamine (LSD-25), and 5-hydroxytryptamine; it is 36°C. for chlorpromazine, and about 20°C. for 2,4-dinitrophenol. In curarized dogs it is about 24°C. for pentylenetetrazol (96). The critical temperature depends upon the effects of the drugs on heat production and on heat loss.

The various types of relationships between the toxicity of a drug and environmental temperature may be graphically represented by the lines drawn in Figure 1,*A, B,* and *C*. The most common relationship is that given in Figure 1,*A,* in which minimum toxicity occurs at some temperature (Y) usually lying between room temperature and thermal neutrality (i.e., 17° to 30°C.). Toxicity increases at temperatures both below and above this point.

This relationship holds for chlorpromazine (9), α-naphthylthiourea (76), BAL (75), strychnine, atropine, digitalis glycosides, and many other drugs (57). Toxicity at temperatures X and Z may be equal, or either may exceed the other, depending upon the exact temperatures used. The relationship shown in Figure 1,*B*, i.e., continuously increasing toxicity with increasing temperature, has been found for dinitrophenol (33, 57), cortisone (91), ephedrine, methacholine, and other drugs (57). This relationship is less common than that shown in Figure 1,*A*. That in Figure 1,*C* is still less common but appears to obtain over a wide temperature range for procaine (57, 85), caffeine, pentylenetetrazol, and some other drugs (44, 57). It may, however, represent only the right-hand part of the U-shaped curve of Figure 1,*A*.

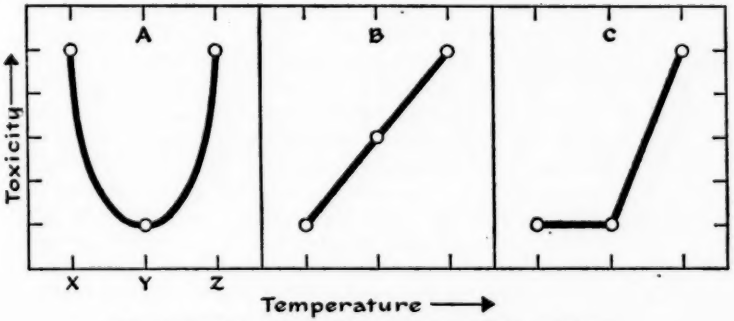

FIG. 1. Possible relationships between toxicity of drugs and environmental temperature.

In most published reports, the temperature range studied was not adequate to permit classification of the data into one of the relationships shown in Figure 1. It is well worth noting, however, that the relationships between toxicity and temperature which would be represented by the reciprocals of the lines drawn in Figure 1 have not been found.

Central nervous system depressants.—Synthetic analgetics [methadone, meperidine, and Hexalgon (1-piperidine-3,3-diphenyl-4-hexanone)] are about three times as toxic at an environmental temperature of 29° as at 18°C. when given subcutaneously to mice (42). This is not a result of difference in rate of absorption, since the same difference in toxicity was found at the two temperatures when methadone was administered intravenously. These authors (43) tried to relate toxicity to the concentration of the drug in the brain. Using an LD_{50} of methadone, it was found that the brain contains four times as much of the drug at an environmental temperature of 18° as at 29°C. Thus, there is a high brain concentration and a lower toxicity at the low temperature. But body temperature may fall to as low as 22°C. at an environmental temperature of 18°C., and, as the authors

suggest, the temperature of the brain may be lowered enough to protect the mice even though the concentration of the drug in the brain is high.

Komlós & Földes (59) reported that sleeping time of rats given hexobarbital was longer when either histamine or serotonin was used with it and that the increase in sleeping time was greater at an environmental temperature of 30° than at 20°C. Hexobarbital alone produces slightly longer sleeping time at 20° than at 30°C.

Berti & Cima (9, 10) administered chlorpromazine subcutaneously to mice. The relationship found conforms to that shown in Figure 1,*A;* at 28°C. the LD_{50} was 350 mg./kg., at 13°C. it was 12 mg./kg., and at 38°C. it was 30 mg./kg. At the higher temperature, death followed violent convulsions, whereas at the low temperature death followed prolonged central depression. Similar results were obtained by Srámková, Votava & Buda (98). Mice and rats pretreated with chlorpromazine cool much more rapidly than controls when placed in a cold environment (70, 95, 107), and rats also lose their ability to regulate body temperature in the presence of a heat load (14, 95). This "lability" of body temperature is much less evident in larger animals (19, 63). Thus, one might attribute the increased toxicity of chlorpromazine in mice at both high and low environmental temperatures to such sufficient interference with temperature regulation that death is partly attributable to hypothermia or hyperthermia. In mice, chlorpromazine produces a fall in body temperature at an environmental temperature of 22°C. (64). This effect is dependent upon dose; no fall occurs with doses under 1.5 mg./kg. Chlorpromazine also prolongs sleeping time after barbiturate injection at an environmental temperature of 22°C. It is well known that lowered body temperature prolongs sleep produced by barbiturates (32), but this result with chlorpromazine is not solely a result of decreased body temperature because it was found that at an environmental temperature of 36°C. where body temperature could not fall, the drug still prolonged sleeping time, probably by direct central depression. Reserpine (64), 5-hydroxytryptamine, and epinephrine (30) are similar to chlorpromazine in that they produce a fall in body temperature and prolong sleeping time with barbiturates or chloral hydrate.

Local anesthetics.— It has been known since 1937 that procaine is more toxic in mice at temperatures above 24°C. than at temperatures between 7° and 24°C. However, Pulewka & Berkan (85) showed once again that procaine and 3-dimethylamino-1,2-dimethylpropyl *p*-aminobenzoate (Tutocaine) are more toxic for mice at an environmental temperature of 37° than at 25°C. or below, whether the index of toxicity is death or convulsions. Although accelerated absorption occurs, this is compensated for by increased rate of excretion. Investigations were continued on rats because, in Istanbul, a thermometer small enough to measure the body temperature of mice was not available. Results on toxicity were similar to those obtained on mice (86). With procaine, rectal temperature of the rats fell at an environmental temperature of 22° and rose at an environmental temperature of 37°C.

The difference in body temperature was about 5°C. The animals at 37°C. became so hyperthermic (rectal temperatures were about 42.7°C.) that, we believe, death could have resulted from this alone, and it is unnecessary to propose, as the authors do, a further effect of procaine of producing anoxia. Inhibition of thermoregulation is clearly an effect of the drug.

Parasympathomimetic drugs.—The anticholinesterase, parathion (O,O-diethyl O-*p*-nitrophenyl thiophosphate), was administered intraperitoneally to mice kept at environmental temperatures of 35.6°, 22.8°, and 15.6°C. (7). It was about twice as toxic at 35.6° as at 22.8°C. and symptoms occurred much faster at 35.6°C. The results at 15.6°C. were complicated by delayed mortality.

Another anticholinesterase, sarin, has been administered intramuscularly to rhesus monkeys (26). It is more toxic at an environmental temperature of 38° than at 25°C. Body temperature fell at the lower temperature and rose at the higher temperature. In other experiments the monkeys were exposed to sarin vapor in a box that excluded their heads. Again the drug was more toxic at 38° than at 25°C., and there were small but definite differences in rectal and skin temperatures. This evidence suggests that, since muscle and skin blood flow are greater at 38°C., increased rate of absorption is responsible for the greater toxicity at high environmental temperatures.

Hormones.—Mice at environmental temperatures of 36°, 27°, and −6°C. were given 0.5 mg. cortisone acetate intramuscularly every 48 hours for eight days (91). Mortality was highest at 36° and lowest at −6°C. The control animals did not survive at −6°C., but those given cortisone lived. Cortisone administered with diphosphopyridine nucleotide is more toxic in mice at environmental temperatures of 37° and 7°C. than at room temperatures (40).

Miscellaneous.—The toxicities of H_2SO_4 mist (84) and CO (53) were reported to be greater at about 0°C. than at room temperature. But the toxicity of NO_2, which produces death from pulmonary edema, increases about 25 per cent with an 11°C. increase in environmental temperature (37). The greater toxicity was probably a result of larger respiratory volume at the higher temperature.

In mice given tetanus toxin, survival time decreases with increase in environmental temperature from 10° to 35°C. (49). Histamine is 25 times more toxic in mice at 37° than at room temperature (20).

At environmental temperatures of 0° and 37°C. the toxicity in mice of the venom of the Australian red back spider is 100 times as great as at room temperature (18° to 24°C.) (109). At the lower environmental temperature, death may result from hypothermia added to the effect of the venom, since body temperature fell 15°C. in three hours after injection.

Stahnke (99, 100) described the use of ligature and cryotherapy in the treatment of venomous bites and strings. Death, or extensive tissue destruction, can be prevented by immediate application of a very tight ligature

close to the site of injection of the venom and rapid cooling of the bitten hand or foot in crushed ice. Tissue temperature is thus reduced from 37°C. to about 4° to 7°C. while the body is kept warm. Cooling is continued while the ligature is released and absorption of the neurotoxic factor is slowed enough to prevent death. Bata & Vukobratovic (8) studied the effect of local cooling and hypothermia (18° to 20°C.) on the toxicity of viper venom in rats. Death was delayed, but not prevented, by either treatment. They state (in desperation?) that local cooling followed by amputation gives 100 per cent protection!

Body Temperature

There are several distinct, but interrelated, effects of body temperature on the action of drugs which, if not recognized, may make interpretation of data difficult and suggest contradictions where there are none. (*a*) The effect of body temperature on toxicity. Toxicity and duration or intensity of action may be affected quite differently by change in body temperature. For example, during hypothermia the toxicity of pentylenetetrazol is unchanged, while its duration of action is increased several hundred times. (*b*) The effect of temperature on duration or intensity of action. Changes in these aspects of the action of drugs during hyperthermia or hypothermia are frequently the result of the algebraic sum of the effect of temperature on absorption and on excretion or detoxification. (*c*) The effect of temperature on a process or function which reveals an action of a drug not apparent at normal body temperature. For instance, a concentration of acetylcholine, which has no effect on isolated rabbit atria at 37°C., re-establishes contraction abolished by cooling (74).

Central nervous system depressants.—The effects of central nervous system depressants added to those of hypothermia are complex but interesting. The well-known depression of the central nervous system caused by hypothermia is preceded by a hyper-responsiveness (58). The hyper-responsiveness is not caused by increased excitability, but by more extensive participation of interneurons in reflex action and by repetitive discharge of neurons following stimuli normally producing single responses. Certain depressants, such as ethyl alcohol (104) or benzene (97), when applied to peripheral nerves at low temperatures, produce repetitive firing, but neither cold nor the narcotic alone produces it. There is as yet no simple explanation for this, but an hypothesis based on membrane inductance has been proposed (97).

There have been very few investigations of the actions of central nervous system depressants at low temperatures, although they are used extensively for the induction of hypothermia. Chlorpromazine is one of the principal constituents of the *cocktails lytiques* used by the French for this purpose. When rats are given chlorpromazine before cooling, they cannot be cooled to lower rectal temperatures before cardiac arrest occurs

than without the drug (69). However, when rats are kept at a rectal temperature of 23°C., chlorpromazine prolongs survival 2.5 times (69). The toxicities of chlorpromazine in rabbits (6) and of pentobarbital in rats (94) increase during hypothermia.

Investigations of the detoxification of central nervous system depressants at low body temperatures have been made. Detoxification is, of course, an important determinant of the duration and intensity of action of these substances. The effect of temperature on detoxification is well illustrated by the studies of Rink *et al.* (88), who perfused isolated rabbit liver with blood containing morphine. Reducing the temperature from 37° to 24°C. increased biological half-time of disappearance of free morphine from the perfusate by 25 times.

The rate of oxidation of ethyl alcohol is reduced during hypothermia (50) as previous work had already shown (29, 31). Hypothermia, induced after administration of alcohol to rabbits, decreased both rate of absorption and rate of oxidation of the alcohol (50). Lowering the temperature of the chick embryo from 38° to 26°C. decreased the rate of oxygen consumption and of alcohol oxidation equally (28).

Central nervous system stimulants.—Bogdanovic (15) reported that in rats cooled to 16° to 18°C. by the Giaja method, the toxicities of cocaine and picrotoxin were increased, whereas that of strychnine was decreased [cf. also (35)]. He suggests that perhaps this difference is related to the different levels of the nervous system which these drugs stimulate. The opposite results were reported earlier on cats anesthetized with barbital (cf. 31). The toxicity of pentylenetetrazol, which appears to act at numerous levels, was not altered by hypothermia (15).

Owens (83) compared the effects of electrical stimulation and chemical convulsants on electroencephalogram (EEG) patterns at different body temperatures. Cats immobilized with succinylcholine were used. A given electrical stimulus (applied to the motor cortex) which produced seizure patterns at normal body temperature failed to do so at body temperatures below 26°C. But pentylenetetrazol or strychnine, injected intravenously in doses which produce minor changes in EEG at normal body temperature, produced very much greater response at low body temperatures. For example, a dose of pentylenetetrazol that lasted eight seconds at 37° lasted 18 to 20 minutes at 26°C.

Parasympathomimetic drugs.—There is much evidence now that the action of acetylcholine on the heart is greatly influenced by temperature. Turpaev (105), using electrically stimulated frog hearts at temperatures from 2° to 40°C., showed that acetylcholine, in a concentration of $1 \times 10^{-7} M$, had the maximum effect on amplitude at 18°C., and less effect above and below this temperature. At 3°C., even 1000 times this concentration had no effect. The heart of *Citellus* also did not respond to acetylcholine at 3°C., but the maximum sensitivity of this heart preparation was at 35° to 38°C.

Marshall & Vaughan Williams (74) studied the influence of acetylcholine on the excitation of isolated rabbit atria at low temperatures. This careful work showed that cooling to 14° to 20°C. abolished all activity except non-propagated pacemaker potentials. At these temperatures acetylcholine (10^{-6} to 10^{-8} g./ml.) produced propagated action potentials and contraction. This was potentiated by physostigmine and abolished by atropine (these drugs had no effect alone), and the doses of acetylcholine used had no effect at 30°C. The slowing of the dog heart produced by intracoronary acetylcholine (79) is greatly prolonged by lowering the body temperature to 24° to 27°C. König (60) found that the expected decrease in rate of the frog heart produced by acetylcholine could be prevented by cooling the sinus to 10°C., but when it was cooled to 5° or 1°C., acetylcholine increased the rate. This is rather surprising, unless differential cooling or block resulted in a ventricular rate independent of the cooled sinus venosus, but the author does not discuss this. Rindani & Merchant (87) found no slowing of the frog heart by acetylcholine below 20°C.

Sympathomimetic drugs.—It has been known for over 50 years that at low body temperatures the response to epinephrine is prolonged and that the effect which it produces on rate and amplitude of contraction of the heart is reduced. Although recent experiments have provided additional data, they have not greatly clarified the reasons for the observed differences in effects at low temperatures. Kruta (61) studied the effect of epinephrine on cooled perfused atria from various species of animals and found that the rate of guinea pig and rabbit atria increased more than that of rat atria. Hearts removed from rabbits cooled to 23°C. and then perfused at 23°C. were more sensitive to epinephrine than hearts removed from normal rabbits and perfused at 38°C. (78). A fixed dose (5 μg.) of epinephrine produced progressively less effect on heart rate and strength of contraction of perfused rabbit hearts as temperature was lowered from 40° to 20°C. (101). Booker (17) compared the effects of temperature on the action of epinephrine and norepinephrine on isolated perfused guinea pig heart. The threshold to both drugs was elevated 10 times by reducing the temperature from 37° to 25°C. The effect was also of longer duration at the low temperature. Low concentrations of cocaine that had a positive inotropic effect on the heart at 37°C. had only a negative inotropic effect at 25°C. (16).

The action of epinephrine is more complicated in cooled whole animals (cf. 21). Szekeres & Lénárd (101) found that in the cat a dose of 5 μg. produced less effect on heart rate, heart contraction, and blood pressure as the body temperature was reduced to 20°C., and that effects were greatly prolonged at the lower temperatures. At body temperatures of 15°C. this, or larger, doses had no effect on the heart rate of vagotomized anesthetized rats (22). These differences cannot be attributed to slowed circulation during hypothermia. Using a totally denervated rat heart and larger doses of epinephrine, Kayser (54) studied the heart rate with and without epine-

phrine at various temperatures. He found epinephrine less effective at lower temperatures. Calculated from the Arrhenius equation, μ was the same for the normal heart as for the denervated heart with epinephrine. The coefficient was smaller for the denervated heart without epinephrine.

Cotten & Brown (24) administered small doses of pressor amines intravenously to dogs at 36° and about 24°C., and measured the effects on the contractile force of the heart, systolic and diastolic pressures, and heart rate. The duration of action of all the drugs was usually about twice as long at the lower temperature. Effects of epinephrine, ephedrine, methamphetamine, phenylephrine, and methoxamine were either unchanged or moderately reduced during hypothermia. However, the effects of levarterenol on contractile force and blood pressure were much smaller during hypothermia than at normal body temperature.

Malméjac and co-workers have published many repetitious papers (three of which are cited here) describing effects of infusion of 1 to 2 µg./kg./min. of epinephrine in hypothermic animals. The epinephrine is reported to restore conduction in the heart (71), counteract ganglioplegia (72), and increase electrical activity of the cerebral cortex (73).

Neuromuscular and ganglionic blocking agents.—The excellent work of Holmes, Jenden & Taylor (46), using strips of rat diaphragm stimulated directly and via the motor nerve, describes the action of tubocurarine at different temperatures. Seperate equilibrium and kinetic experiments were performed to distinguish between factors affecting the activity of the drug and the rate of action. In equilibrium experiments it was shown that a reduction of temperature from 40° to 26°C. reduced neuromuscular blocking action. Further decrease in temperature potentiated blocking action. Then, using a dose large enough to block neuromuscular transmission at all temperatures, they found that the speed of paralysis is determined by rate of diffusion of the tubocurarine to the site of action. When the logarithm of the velocity is plotted as a function of the reciprocal of the temperature, a straight line results, and μ is 5100 (Q_{10} about 1.3). Removal of the drug from the site of action is also determined by diffusion. However, according to Segre (92) the action of tubocurarine on the diaphragm does not follow the Arrhenius equation.

Using cats anesthetized with chloralose and pentobarbital and cooled, Bigland *et al.* (13) studied the action of tubocurarine on the contraction of the tibialis muscle after stimulation of the sciatic nerve. Various types of experiments were used such as keeping one leg warm while the other was cooled, keeping the body warm while the leg was cooled, etc. They found, as did Holmes, Jenden & Taylor (46), that tubocurarine is a less effective blocking agent at 25° to 30° than at 38°C. Also, the duration of block was not affected by decrease in temperature. In further experiments (13), other blocking agents, succinylcholine and decamethonium, which act by depolarizing the motor end plate, were studied. It was found that with a given dose

of either drug, the duration of action was much longer at low temperatures (the duration was four times as long at 31° as at 36°C.), and cooling increased the magnitude of the blockade. Thus, decrease in temperature has an opposite action on blockade by these two agents and blockade by tubocurarine. The effect of temperature on blockade by succinylcholine and decamethonium is about the same; yet the former is rapidly hydrolyzed by plasma pseudo-cholinesterase whereas the latter is excreted. Thus, the effect of cooling in increasing block is probably not the result of slowed enzyme action. Both drugs depolarize motor end plates, and recovery from depolarization is greatly slowed by decrease in temperature (27). This is probably the explanation of the more effective block at low temperatures. It might be presumed that lowering the temperature should make acetylcholine more effective (since succinylcholine acts like acetylcholine), thus reducing the effectiveness of drugs that compete with acetylcholine. Since the action of tubocurarine depends on competition with acetylcholine, it would be less effective as a blocking agent at low temperatures; this was the experimental result. Much of this work on blocking agents was repeated on human subjects with the same results (18).

Histamine and antihistaminics.—The effect of temperature on the release of histamine seems to depend upon the nature of the histamine liberator. Monger & Schild (77) found that the release of histamine from guinea pig lung by egg albumen *in vitro* was maximal at 40° and absent at 20°C. In hypothermic rats at 14° to 16°C., less histamine is released by intraperitoneal dextran than in controls (108). Release of histamine by intradermal dextran was also decreased by cooling. In the excellent work of Halpern, Neveu & Brannellec (38), blood histamine was measured in rats cooled by the Giaja method to a body temperature of 15°C. After administration of basic amines [*p*-methoxy-N-methyl-phenethylamine (48/80) and 1935L] blood histamine was higher in hypothermic rats than in controls. This was probably a result of decreased rate of destruction of histamine. But the liberation of histamine by dextran was completely blocked by hypothermia. This is good evidence that, at least in hypothermia, there is a dual mechanism of liberation of histamine which is influenced by the physicochemical nature of the histamine-liberating substance.

The effect of temperature on the antagonism between histamine and pyrilamine and histamine and diphenhydramine was studied in guinea pig ileum (93). Gillespie showed that hypothermia decreases the volume and acidity of gastric secretion induced by histamine in the cat (36). However, when gastric acid secretion was recorded continuously in the rat, moderate cooling increased the response to histamine and moderate heating decreased it (34).

Cardiac glycosides and aglycones.—Digitalis and related compounds are less toxic and less effective in homeotherms when body temperature is below normal. Recent evidence is in complete agreement with much older data

on frogs, etc. (cf. 31). The dose of digilanid necessary to produce cardiac arrest in cats is twice as great at 25° as at 37°C. (89). In hypothermic dogs, more ouabain (5) and more deslanoside (80) were required to produce typical digitalis effects than in normothermic animals. Ouabain was less effective in increasing the contractile force of the heart in hypothermic dogs than in normothermic dogs (24).

Saunders & Sanyal (90) used electrically stimulated strips from guinea pig ventricle to study the action of ouabain at temperatures from 17° to 37°C. Change in temperature had a negligible effect on the rate of onset of action of ouabain, but the positive inotropic effect increased with increasing temperature. The relatively small effect at low temperatures may be caused by low intracellular potassium at low temperatures.

Metabolic inhibitors and stimulants.—In dogs cooled to 20°C., the chemoreceptors in the carotid body still respond to cyanide (81). More cyanide is required to produce death of hypothermic (28°C.) dogs than of controls (51).

During hypothermia the behavior of a young mammal and a hibernator differs from that of an adult homeotherm, the two former continuing to respire at lower body temperatures. It is well known that glycolysis is much more important during the first few days after birth than in the adult, and one may ask if the resistance to cold of the newborn homeotherm and the hibernator is caused by a special property of the nervous tissue of the center or of the myocardium. Kayser attempted to answer this by determining the effect of metabolic inhibitors and a stimulant on QO_2 of these tissues at different temperatures. Malonate depressed the QO_2 of hamster brain slices less than the QO_2 of rat brain slices at 38°, 30°, 23°, and 16°C. (56). Depression of rat brain QO_2 was similar at all temperatures, but there was less inhibition of hamster brain QO_2 at the lower temperatures. Dinitrophenol produced the same inhibition at all temperatures [this result differs from that of Fuhrman & Field (cf. 31) and Locker (see below)]. Iodoacetate depressed the QO_2 of hamster heart less than that of rat heart at temperatures from 16° to 38°C. (55).

Locker tested the effect of various concentrations of 4,6-dinitro-*o*-cresol on the QO_2 of tissues in Ringer-bicarbonate (without substrate) at different temperatures from 17.5° to 42.5°C. Two types of response to 4,6-dinitro-*o*-cresol were found. (*a*) In frog and toad liver (65, 68) maximum stimulation of QO_2 occurred at about 32.5°C. or higher, and stimulation decreased (or became inhibition) with decrease in temperature. (*b*) In the liver of the mouse, rat, and guinea pig and skin of frogs (66, 67), maximum stimulation occurred at or below 22.5°C. and decreased with increase in temperature. Locker interprets his data according to the theories of enzyme inhibition developed by Johnson & Eyring (52), based on the concept that enzymes exist in equilibrium between a catalytically active and inactive form.

Miscellaneous drugs.—Hasegawa & Matsuda (39) and Takahashi &

Matsuda (102) determined the time required for various drugs (digitalis, strychnine, acetylcholine, etc.) to produce characteristic effects on toad and fish hearts and perfused toad limbs at various temperatures. Values for Q_{10} were usually between 1.5 and 2.5. They state that these coefficients reflect differences in adsorption of the drugs and come to the astonishing conclusion that this explains all differences in actions of drugs, and that it is unnecessary to assume any chemical changes in them or any changes in cell metabolism.

The toxicity of dipicrylamine in fish is the same at 20° and 3°C., but the time required to produce the first signs of poisoning after injection is longer at the lower temperature (103). When rat uterus was cooled *in vitro* to 23°C. with oxytocin added to the fluid around it, the phasic contractions associated with action potentials were suppressed (62). Cooling the isolated guinea pig ileum to about 20°C. abolishes the emptying phase of the peristaltic reflex; introduction of small amounts of 5-hydroxytryptamine into the lumen restored peristalsis (12).

The hyperglycemia produced in rabbits by injection of pentobarbital at a body temperature of 38° fails to occur when this drug is injected at 27°C. (1). The same authors also confirm older observations that insulin hypoglycemia is greatly delayed or prevented by cooling.

The antidiuretic action of exogenous posterior pituitary hormone is reported to be absent in hypothermic rats (47). This hormone increases water uptake through the skin of frogs, and the effect is abolished by cooling the frogs to 1°C. (48).

Olsson, William-Olsson & Lagergren (82) found that the elimination of heparin from plasma is exponential in both normothermic and hypothermic dogs. The average time for 50 per cent decrease in plasma heparin was 63 minutes at 36° and 128 minutes at 29°C.

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BIOCHEMICAL EFFECTS OF DRUGS[1]

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Certain representative biochemical effects have been selected for this first volume of the *Annual Review of Pharmacology*. Where a biochemical effect appears pertinent to an understanding of the drug's mechanism of action, we have framed our discussion accordingly.

Drugs Influencing Amine Metabolism

Reserpine and other Rauwolfia alkaloids.—In the past several years, studies on the various biochemical effects induced by reserpine and related Rauwolfia alkaloids have stimulated the interest of biochemists as well as pharmacologists. Classical pharmacological techniques were employed initially to investigate the mechanisms of the central and peripheral pharmacologic effects induced by reserpine (depression, hypotension, hypothermia, bradycardia, etc.). Early conclusions were that reserpine acts primarily by reducing the activity of the central sympathetic regulating centers (1) or by producing a central block or inhibition of afferent nerve impulses which normally stimulate sympathetic activity (2).

The idea that reserpine might release certain body amines arose from observations that serotonin (5-hydroxytryptamine) administration to mice produces certain effects similar to those of reserpine (3, 4, 5): sedation, potentiation of hypnotics, and blockade of this action of both reserpine and serotonin by the serotonin antagonist, lysergic acid diethylamide. Shore *et al.* (5) observed that administration of reserpine to dogs caused a marked increase in the urine levels of 5-hydroxyindoleacetic acid, a metabolic product of serotonin, suggesting that serotonin had been released from its stores and undergone metabolism. This view was verified by direct analysis of tissue serotonin levels which showed that reserpine treatment lowers the serotonin content of intestine (6), brain (7, 8), platelets (9, 10), and other tissues including rat mast cells (11).

From an examination of the effect of several Rauwolfia alkaloids on brain serotonin levels, Brodie *et al.* (12) concluded that only those which elicit a sedative response affect the levels of serotonin in brain. Hess *et al.* (13) demonstrated that the depressant effects of reserpine persist long after the time that any appreciable levels of reserpine remain in the brain, whereas the pharmacologic effects parallel temporally the decline in serotonin levels.

[1] The survey of the literature pertaining to this review was concluded in July, 1960.

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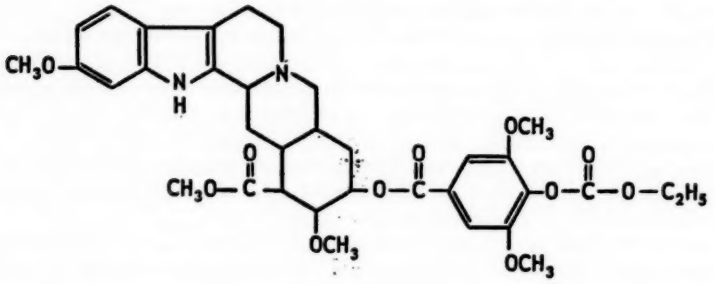
Sheppard *et al.* (14) reported the presence of trace amounts of radioactive material in the brain long after injection of reserpine-C^{14}, and interpreted their results as an indication that reserpine per se, rather than changes in brain amine levels, may be responsible for the persistent effects of a single dose of reserpine. Their results show, however, no correlation between levels of radioactive material and pharmacological effects.

Studies in man have shown that clinically effective doses of reserpine are effective in altering blood serotonin levels (15). A prolonged biochemical "lesion" in tissues was evidenced by the demonstration that after reserpine administration, platelets and brain fail to take up as much added serotonin as do normal tissues (16).

The inference that brain serotonin is important in the pharmacologic action of reserpine (17), made on the basis of the above mentioned find-



Su 3118

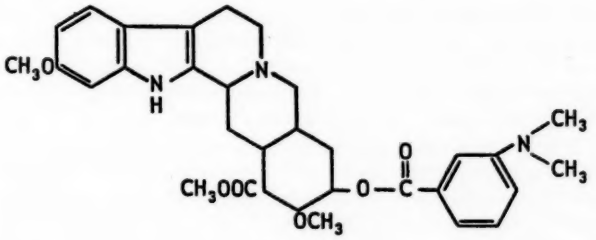
FIG. 1

ings, was challenged by the observation that reserpine also releases catecholamines from tissues including brain (18, 19, 20), heart (20, 21), ganglia (22), arteries (23), and blood (24). Furthermore, almost identical dose and temporal relationships are evident in the effects of reserpine on serotonin and norepinephrine levels in brain (25). More recently, the identification of dopamine (hydroxytyramine) in brain was followed by the observation that reserpine treatment leads to the release of this amine, too (26).

Persuasive evidence is now available that the depletion of norepinephrine from peripheral sympathetic nerve endings is largely responsible for the lowered peripheral sympathetic tone induced by reserpine. Thus it has been demonstrated that after depletion of tissue norepinephrine by reserpine or similarly acting congeners, animals no longer show the usual peripheral responses to electrical or chemical stimulation of sympathetic nerves, although the response to injected norepinephrine is unchanged or potentiated (19 to 22, 27). One semisynthetic Rauwolfia alkaloid, Su 3118 (Fig. 1), exerts an effect primarily on peripheral norepinephrine stores over a wide

dosage range, although higher doses of the drug also cause depletion of amines from the brain with a consequent central depression (28).

To ascribe the central effects of reserpine to a single amine has proved to be a more difficult problem. Arguments that the central depressant effects of reserpine are caused by depletion of brain catecholamines have been based on observations such as the ability of injected dihydroxyphenylalanine and the inability of injected 5-hydroxytryptophan to reverse the depressant effects of reserpine (29), or on attempted differential depletion of the amines by certain drugs (30). More recently, however, evidence has been accumulating that the depressant effects of reserpine are indeed more closely related to changes in brain serotonin levels than to brain catecholamine levels. For example, by the use of another semisynthetic reserpine analog, Su 5171 (Fig. 2), which alters brain norepinephrine levels longer than brain serotonin levels, it has been shown that the depressant effects of the alkaloid closely parallel changes in the level of serotonin, but not norepinephrine (31).



Su 5171

FIG. 2

Stronger evidence for the primary importance of brain serotonin in the central actions of reserpine has been obtained from experiments in which the alkaloid has been administered to cold-exposed animals (32, 33). Under this condition, reserpine lowers brain norepinephrine levels, but has little effect on brain serotonin levels and produces no sedation until the brain serotonin levels finally decline (33).

In studies on the mechanism of reserpine-induced release of serotonin in a model system of rabbit platelets *in vitro*, Carlsson *et al.* (34) found that the releasing activity of reserpine is remarkably potent, one molecule of the alkaloid effecting the release of hundreds of serotonin molecules. In spite of this, platelets are not broken by the drug (9). An analysis of the kinetics of serotonin uptake by platelets in the presence and absence of reserpine has suggested that the drug interferes with a serotonin transport mechanism which maintains serotonin levels within the platelet against a concentration gradient (35).

Tetrabenazine and other benzoquinolizines.—Another series of compounds that markedly lower brain serotonin and norepinephrine levels is the benzoquinolizine series, of which tetrabenazine has been the most widely investigated. Pletscher and co-workers (36) found that this drug and its active congeners release brain serotonin and norepinephrine and produce central depression similar to that induced by reserpine. Unlike the Rauwolfia alkaloids, however, the benzoquinolizines are relatively short acting in their actions on brain amines and in producing sedation. The short duration of action of these compounds suggests that they may act "reversibly," rather than "irreversibly" as does reserpine.

Tetrabenazine seems to have little effect on peripheral amine stores (37), although a recent report holds that heart norepinephrine levels are lowered by the drug (38). Consistent with the relative absence of peripheral norepinephrine release is the observation that, unlike reserpine, tetrabenazine has little or no effect on blood pressure (39).

In spite of their differences, tetrabenazine and reserpine appear to act in the brain at the same receptor sites, since it has been demonstrated by Quinn *et al.* (37) that treatment of animals with tetrabenazine just prior to administration of reserpine markedly lessens the duration of action of reserpine. The results of this experiment provide further evidence for the "irreversible" nature of reserpine action, because tetrabenazine is of itself short-acting, but remains in the brain for a time sufficient to inhibit the action of reserpine at its receptor sites during the even briefer physical presence of reserpine in the brain.

Effect of reserpine on histamine levels.—In addition to affecting serotonin and catecholamines in tissues, reserpine lowers histamine levels in rabbit blood (40), but not in other rabbit tissues (41). The alkaloid does not lower histamine levels in rat skin (mast cells), although serotonin is released from this tissue (11). The effect on histamine levels in rabbit blood, like the effects on other amines, seems to be confined to sedative Rauwolfia alkaloids since an almost inactive one, methyl reserpate, causes little lowering of rabbit-blood histamine levels (41). Inasmuch as Burkhalter *et al.* (41) found that infusion or injection of serotonin or its precursor causes a lowering of blood histamine levels, there is the possibility that the serotonin released by reserpine in the blood may, in turn, be responsible for the histamine release from rabbit platelets. Surprisingly, neither reserpine nor serotonin effects the release of histamine from rabbit platelets *in vitro,* although serotonin is readily released by reserpine in the same system.

Guanethidine.—The saying, "It never rains but it pours," seems to apply to amine-releasing agents, and a new hypotensive drug, guanethidine, must be added to the growing list. Investigation into the mode of action of this drug has revealed that it causes peripheral effects that, as with the Rauwolfia alkaloids, might be described as "chemical sympathectomy," since the action of the drug renders the sympathetic system unresponsive to

stimuli, although the action of injected norepinephrine is not inhibited [Maxwell *et al.* (42)]. Suggestions that the drug might alter the state of the adrenergic transmitter have been borne out by the observation of Cass *et al.* (43) that guanethidine causes a marked lowering of norepinephrine levels in various peripheral organs, but not in brain or adrenal glands. The rate of norepinephrine depletion of tissues by guanethidine is considerably slower than that observed following reserpine administration, suggesting a difference in the mode of action. It seems possible that guanethidine might act by blocking the synthesis of norepinephrine rather than by a true release process.

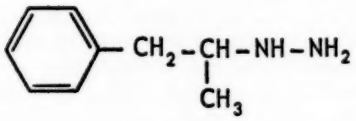
Bretylium, a hypotensive drug which causes a pharmacologic effect similar to that of guanethidine, does not appear to act by depletion of norepinephrine stores, but possibly by blockade of nerve impulses along the postganglionic sympathetic fiber, an action aptly described by Boura & Green (44) as "adrenergic neurone blockade."

α-Methyl-dihydroxyphenylalanine.—Experiments *in vivo* with α-methyl-dihydroxyphenylalanine, an inhibitor of the enzyme which decarboxylates dihydroxyphenylalanine (45, 46) and 5-hydroxytryptophan (47), showed that the drug causes a lowering of serotonin levels in brain and intestine (48) and of norepinephrine levels in brain and peripheral organs (49). In preliminary studies, Oates *et al.* (50) observed hypotension and sedation in hypertensive patients given α-methyl-dihydroxyphenylalanine.

Monoamine oxidase inhibitors.—The close association of interests between the clinical investigator and the basic researcher has seldom been more evident than in studies of the actions of monoamine oxidase inhibitors. Much of the impetus for the clinical trial of these drugs arose from laboratory studies, although many of the discrete pharmacologic effects are seen only in the clinic.

Observations in 1952 that tubercular patients undergoing therapy with iproniazid showed signs of central stimulation (51) led to the abandoning of the drug as a tuberculostatic agent in favor of its congener, isoniazid. In that same year, Zeller *et al.* (52) reported that iproniazid inhibits the activity of monoamine oxidase. The various observations remained unrelated until reports appeared indicating that administration of reserpine to animals pretreated with iproniazid does not cause the usual depressant effect of the Rauwolfia alkaloid, but instead elicits a marked degree of central stimulation (53, 54, 55). Measurement of the serotonin and norepinephrine content of various tissues, including brain, showed that pretreatment with the monoamine oxidase inhibitor also prevents the usual serotonin- and norepinephrine-lowering effects of reserpine (53, 54, 56).

These observations and the recollection of the central excitation seen in tubercular patients on iproniazid therapy prompted a reinvestigation of the clinical usefulness of iproniazid, but this time as a central stimulant to combat mental depression (57). The resulting favorable clinical results and the assumption that the antidepressant effects were associated with the mono-



JB 516

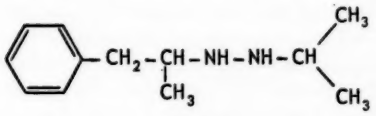
FIG. 3

amine oxidase blocking action of iproniazid led to the rapid synthesis and clinical trial of a number of other monoamine oxidase inhibitors.[3]

Attempts to elucidate in animals the mechanisms involved in the central stimulant effect seen in patients have led to observations that pretreatment with monoamine oxidase inhibitors not only reverses reserpine-induced depression and prevents the decrease in the levels of brain catecholamines and serotonin, but that the inhibitors cause a marked increase in the concentrations of these amines in brain (54, 60 to 64). Thus, a single large dose of iproniazid, or JB 516 (Fig. 3), causes a two- to threefold increase in serotonin and norepinephrine levels in rabbit brain. There exists a species difference with regard to this effect; rabbits, rats, mice, and monkeys show an elevation in both serotonin and norepinephrine brain levels after treatment with monoamine oxidase inhibitors, whereas dogs and cats show an elevation only in serotonin levels (63). Pretreatment of dogs with a monoamine oxidase inhibitor, however, does prevent the usual lowering of brain norepinephrine levels by reserpine (65).[4]

[3] Doubt has been expressed that the clinical antidepressant action of monoamine oxidase inhibitors is related to blockade of the enzyme; instead, it is thought that it is attributable to some other action of the drugs. However, the primary criterion for selection of the drugs for further investigation has been their ability to block monoamine oxidase *in vitro* and *in vivo*. One report (58) holds that 1-benzyl-2-methyl-5-methoxytryptamine (BAS), though a sedative agent in man, inhibits monoamine oxidase, a conclusion based on indirect evidence. Direct measurement of the ability of BAS to block brain monoamine oxidase *in vivo* or *in vitro*, however, has demonstrated that this agent has little or no monoamine oxidase inhibitory activity (59).

[4] The ability of monoamine oxidase inhibitors to reverse the depressant effects of reserpine and to prevent the usual rapid decrease in the brain content of catecholamines and serotonin following reserpine administration was originally interpreted as evidence that the amines were released by reserpine from their binding sites, but that since they could not be acted upon by the inactivated monoamine oxidase, they remained in the brain in a free form (53). This view has been challenged by suggestions that the monoamine oxidase inhibitors not only block the enzyme, but prevent release of the amines as well. Recently, however, Spector *et al.* (66) have demonstrated that monoamine oxidase inhibitors do not prevent the reserpine-induced release of platelet serotonin or the release of brain serotonin and catecholamines, but appear, instead, to stabilize the liberated amines.



JB 807

FIG. 4

After a single large dose of iproniazid in rabbits, no obvious gross pharmacologic signs can be detected in the animals in spite of the elevated brain amine levels, but prolonged daily injections of the drug provoke a degree of excitation and peripheral sympathomimetic signs together with a further elevation of the brain amine levels (63, 64).

Attempts to associate these pharmacologic signs with one or the other of the brain amines have not yielded clear-cut answers. There is no obvious relationship between the excitatory effects and the degree of elevation of brain serotonin levels. A better association exists in the case of brain norepinephrine levels. Spector *et al.* (63) observed that with several monoamine oxidase inhibitors, excitation in rabbits did not occur until brain norepinephrine levels reached a critical value. Upon discontinuance of the drug, the excitation ceased when the brain norepinephrine levels decreased, even though the serotonin levels were still elevated. The picture is by no means clear, however, as one monoamine oxidase inhibitor, JB 807 (Fig. 4), closely related to JB 516, causes a marked increase in the level of both brain amines, but does not produce detectable excitatory effects in rabbits (64).

It has not been possible to produce these pharmacologic effects by administration of monoamine oxidase inhibitors to dogs or cats (63). In these species, as pointed out before, although a marked rise in brain serotonin levels follows treatment with the drugs, no rise in brain norepinephrine levels occurs. These results suggest again a possible association between central stimulation and elevated brain norepinephrine levels.

Metabolism of catecholamines.—The influence of monoamine oxidase inhibitors on the metabolism of norepinephrine in the brain has led to the hypothesis that the major pathway of catecholamine metabolism in brain is via oxidative deamination by monoamine oxidase (67). This conclusion might seem at variance with studies on the fate of catecholamines injected into the bloodstream. In the latter experiments, Axelrod *et al.* (68) have shown that the major metabolic route of administered catecholamines involves initially an ether formation by 0-methylation of the 3-hydroxyl group. It seems clear, however, that both monoamine oxidase and catechol-0-methyl transferase are of utmost importance, each in its own domain. Monoamine oxidase is probably responsible for metabolism of catecholamines within tissues and for regulation of the tissue stores of these

amines, whereas 0-methyl transferase appears to be mainly responsible for the "detoxication" of catecholamines which find their way into the bloodstream. For example, it has been demonstrated that administration of monoamine oxidase inhibitors does not potentiate the cardiovascular effects of injected epinephrine (69), in contrast to pyrogallol, an inhibitor of the methylating enzyme (70) which potentiates the effects of injected epinephrine (71). However, pyrogallol, even in very large doses, does not elevate catecholamine levels in brain (72), nor does it block the norepinephrine-depleting effect of reserpine even though the activity of the brain-methylating enzyme is blocked by parenteral administration of pyrogallol. Similar results have been obtained from studies on peripheral tissues. Thus, pyrogallol lowers the rate of metabolism of circulating injected norepinephrine but not the rate of metabolism of the injected norepinephrine taken up by heart tissue, whereas monoamine oxidase inhibitors have no effect on the circulating norepinephrine levels but lower the rate of disappearance of norepinephrine taken up by the heart (72).

Association of brain amine changes with convulsive threshold.—Changes in brain amine levels may have a role in the effects of certain drugs on the threshold of electroshock convulsions. A marked facilatory effect of reserpine on experimentally induced convulsions was noted by Chen *et al.* (73) in 1954 before it was known that the drug releases brain amines. More recently, Prockop *et al.* (74) showed that centrally acting monoamine oxidase inhibitors exert a marked anticonvulsant action in rats subjected to electroshock. The onset of the anticonvulsant effect following injection of iproniazid is delayed until the brain amine levels have increased, and the effect persists until the amine levels fall toward normal values. The peak anticonvulsant effect of the more rapidly acting monoamine oxidase inhibitor, JB 516, occurs much sooner than that following iproniazid injection. Thus, there appears to be a marked correlation between brain amine levels and the convulsive threshold. In these experiments, diphenylhydantoin was found to completely block convulsions without causing a concomitant rise in brain amine levels. Thus, the anticonvulsant effect of diphenylhydantoin does not appear to be associated with an alteration of brain amine levels despite the report that very high doses of this drug cause a rise in brain serotonin levels (75).

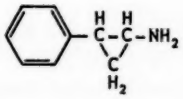
That the facilatory effects of reserpine, on the one hand, and the anticonvulsant effects of the monoamine oxidase inhibitors, on the other, are not caused by the presence of the drugs per se was shown in experiments where monoamine oxidase inhibitors were given to animals previously treated with reserpine (low brain amine levels) or where reserpine was given to animals pretreated with monoamine oxidase inhibitors (high brain amine levels). Again, those animals with low amine levels showed facilitation of convulsions, whereas those animals with high brain amine levels showed a resistance to electrical stimuli (74). Lessin & Parkes (76) have noted that reserpine or tetrabenazine also lowers the survival time of mice

treated with pentylenetetrazol and that pretreatment of the animals with iproniazid inhibits this effect.

Anticonvulsant effects of monoamine oxidase inhibitors also have been observed in man. Grisoni *et al.* (77) observed that iproniazid exerts a normalizing action on the EEG pattern of epileptic patients. More recently, Carter (78) found that isocarboxazid, a potent monoamine oxidase inhibitor, reduced the frequency of grand mal seizures in a high percentage of a group of epileptic patients.

Cardiovascular effects of monoamine oxidase inhibitors.—In addition to the clinically observed antidepressant effects, monoamine oxidase inhibitors have been found to lower standing blood pressure in man (79) and to alleviate the pain associated with angina pectoris (80).

The mechanism of the cardiovascular actions is unclear, especially since the drugs do not appear to cause clear-cut effects in laboratory animals. Several possible pharmacologic mechanisms, including coronary artery dilation, ganglionic blockade, and an inhibition of catecholamine release, have



SKF 385

FIG. 5

been postulated to explain the clinical cardiovascular effects [see review by Zbinden *et al.* (81)]. An elevation in catecholamine levels in peripheral tissues occurs in the heart (82, 83) and autonomic ganglia (84). Elevated blood serotonin levels have been reported in patients and laboratory animals treated with monoamine oxidase inhibitors (85, 86). Presumably all of the extra blood serotonin is localized in the platelets, as in the case with the normal serotonin content of blood.

Other actions and types of monoamine oxidase inhibitors.—Of course, monoamine oxidase inhibitors do not necessarily affect only monoamine oxidase. Indeed, monoamine oxidase inhibitors possessing the hydrazine moiety also inhibit diamine oxidase (87, 88). The action on diamine oxidase appears to be attributable to the hydrazine moiety since aminoguanidine and isoniazid do not block monoamine oxidase but do block diamine oxidase, whereas certain potent nonhydrazine monoamine oxidase inhibitors, such as SKF 385 (Fig. 5), have no action on diamine oxidase (88). Inhibition of diamine oxidase does not appear to be implicated in the hypotensive action of monoamine oxidase inhibitors since SKF 385 also causes postural hypotension (89).

Udenfriend *et al.* (90) have pointed out that monoamine oxidase inhibitors fall biochemically into two categories: irreversible and reversible. The

inhibitors in clinical use, such as iproniazid, irreversibly inhibit the enzyme since inhibition *in vivo* persists long after the drug has disappeared from the body (91, 92), and the inhibitory action *in vitro* cannot be removed by dialysis. Harmaline, a reversible inhibitor, blocks monoamine oxidase only while present in sufficient concentration in the tissues, and its action *in vitro* can be removed by dialysis (90). It appears that the reversible inhibitors occupy the same sites that are altered by the irreversible inhibitors since pretreatment of animals with harmaline blocks the prolonged effect of subsequently administered iproniazid (66, 93).

It should perhaps be pointed out that although the major investigative labors have gone into a study of the effects of monoamine oxidase inhibitors on serotonin and the catecholamines, other amines are affected by these drugs. It has been shown that after administration of these inhibitors to human subjects, several amines, including tryptamine, phenylethylamine, tyramine, and its *o*- and *m*-isomers, appear in the urine (94 ,95). It is possible that one or more of these amines or some as yet undiscovered amine might be important in the action of monoamine oxidase inhibitors.

Relationship Between Vitamin B_6, γ-Aminobutyric Acid, and Seizure-Producing Drugs

During the past 10 years γ-aminobutyric acid has become of considerable importance in pharmacology. The compound appears to be formed and to occur only in the central nervous system. Several functions have been attributed to γ-aminobutyric acid in the brain: (*a*) The compound may act as a neurohumoral agent. (*b*) It may participate in ammonia detoxification via the formation of γ-guanidinobutyric acid. (*c*) γ-Aminobutyric acid may be an important substrate in oxidative metabolism via the following pathway: α-keto-glutaric acid → glutamic acid → γ-aminobutyric acid → succinic semialdehyde → succinic acid. Recent reviews have appeared on the metabolism and postulated roles for γ-aminobutyric acid in brain (96 to 100).

Several lines of investigation have indicated a relationship between vitamin B_6, γ-aminobutyric acid, and seizure-producing drugs. It is known that a dietary deficiency of vitamin B_6 (pyridoxine) in man and animals facilitates seizures. Furthermore, vitamin B_6 antagonists, either structural analogues (deoxypyridoxine, methoxypyridoxine) or carbonyl reagents (isonicotinylhydrazide, semicarbazide, and thiosemicarbazide), are potent convulsants. A possible explanation for the seizures produced by these compounds has come from the observation that vitamin B_6 in the form of pyridoxal phosphate serves as a coenzyme necessary for certain transaminase and decarboxylase reactions involved in the metabolism of γ-aminobutyric acid. Glutamic acid decarboxylase required for the conversion of glutamic acid to γ-aminobutyric acid is found only in brain and is very sensitive to a deficiency of pyridoxal phosphate or to interference with this coenzyme.

Killam & Bain (101) showed that carbonyl reagents markedly inhibit the activity of this enzyme and that the decreased activity is correlated with decreased levels of γ-aminobutyrate in brain.

McCormick & Snell (102) have proposed a mechanism for the action of carbonyl reagents on brain metabolism other than through their direct effect on enzymes involved in amino-acid metabolism. They have isolated pyridoxal phosphokinase from human cerebral cortex and purified the enzyme 200-fold. Both α-methylphenethylhydrazine and isonicotinylhydrazide were found to be extremely effective inhibitors of this kinase. The actual inhibitors, however, are the corresponding hydrazones formed between these hydrazines and pyridoxal. The oxime, hydrazone, and semicarbazone of pyridoxal are all potent inhibitors with affinities for the kinase well over 100 times that of pyridoxal. In view of the low concentration at which the carbonyl reagents inhibit pyridoxal phosphokinase, McCormick & Snell (102) suggested that the seizure-producing effects of these compounds result in part from their capacity to lower or to eliminate production of pyridoxal phosphate. Thus, the carbonyl reagents were postulated to lower brain γ-aminobutyrate levels as a consequence of inhibition of the pyridoxal phosphokinase rather than from inhibition of the glutamic decarboxylase.

Baxter & Roberts (103) reported that the vitamin B_6-dependent γ-aminobutyric acid-α-keto-glutaric acid transaminase of beef brain which catalyzes the conversion of γ-aminobutyric acid to succinic semialdehyde is markedly inhibited by hydroxylamine. They showed that administration of hydroxylamine results in increased γ-aminobutyrate levels in rat brain (104). Eidelberg *et al.* (105) found that administration of hydroxylamine to cats elevates brain levels of γ-aminobutyrate and reduces the duration of electrically induced seizures. The possibility was suggested that the increased γ-aminobutyrate levels may result from a more potent inhibitory effect of hydroxylamine on the γ-aminobutyric acid-α-keto-glutaric acid transaminase than on the glutamic acid decarboxylase. Rindi & Ferrari (106) reported that the seizure-producing compound toxopyrimidine (2-methyl-4-amino-5-hydroxymethyl pyrimidine) lowers the level of γ-aminobutyric acid in rat brain presumably by inhibition of glutamic decarboxylase. Nishizawa *et al.* (107) administered toxopyrimidine and hydroxylamine to mice and measured the activity of both brain glutamic-aspartic transaminase and glutamic decarboxylase and found that when convulsions are induced by toxopyrimidine administration, both enzyme systems are inhibited. When sufficient hydroxylamine is given to cause convulsions, the decarboxylase is inhibited, but the transaminase is not affected unless lethal doses of hydroxylamine are administered. They suggested that the convulsions are related to inhibition of glutamic decarboxylase.

The level of γ-aminobutyric acid in brain depends, presumably, on the relative rates of its formation and utilization by two vitamin-B_6-requiring enzymes, glutamic acid decarboxylase and γ-aminobutyric acid-α-ketoglu-

tarate transaminase. Consequently, drugs that inhibit the decarboxylase decrease the level of γ-aminobutyric acid, whereas those that selectively inhibit the transaminase elevate the level of this compound. Roberts & Eidelberg (96) have pointed out that a causal relationship between changes in seizure susceptibility with changes in γ-aminobutyric acid levels in brain still has not been established.

Interaction of Nicotinamide with Drugs

Kaplan *et al.* (108) reported that the amount of diphosphopyridine nucleotide in mouse liver increases markedly upon the injection of large doses of nicotinamide. Burton *et al.* (109) subsequently demonstrated that the administration of reserpine or chlorpromazine prior to the injection of nicotinamide results in elevated levels of diphosphopyridine nucleotide in liver for a prolonged period of time. Burton *et al.* (110) recently showed a correlation between the ability of reserpine and reserpine derivatives and of chlorpromazine and phenothiazine derivatives to tranquilize animals and their ability to maintain elevated diphosphopyridine nucleotide levels in liver. They noted that sedative drugs such as phenobarbital and ethanol are not able to maintain these high nucleotide levels. In another study, Burton *et al.* (111) demonstrated that nicotinamide acts in mice in conjunction with either reserpine or chlorpromazine to reduce spontaneous activity and to increase the duration of pentobarbital anesthesia. The biochemical mechanisms involved in the effect of reserpine and chlorpromazine in prolonging the increased diphosphopyridine nucleotide levels remain to be established.

Stimulatory Effect of Drugs on Ascorbic Acid Synthesis

Various drugs possessing completely unrelated chemical and pharmacological properties have been shown by Longenecker *et al.* (112), Baumann *et al.* (113), and Burns *et al.* (114) to increase markedly the urinary excretion of ascorbic acid in rats. These drugs include the hypnotics, chlorobutanol (Chloretone) and barbital; the analgesics, aminopyrine and antipyrine; the muscle relaxants, orphenadrine and meprobamate; the antirheumatics, phenylbutazone and oxyphenbutazone; the uricosuric agent, sulfinpyrazone; the antihistaminics, diphenhydramine and chlorcyclizine; the anticoagulant, bishydroxycoumarin (Dicumarol); and the carcinogenic hydrocarbons, 3-methylcholanthrene, 3,4-benzpyrene, and 1,2,5,6-dibenzanthracene. Tracer studies by Horowitz & King (115) and Burns and coworkers (116, 117) showed that this increased excretion results from an accelerated L-ascorbic acid synthesis from D-glucose through the intermediate formation of D-glucuronic acid, L-gulonic acid and L-gulonolactone.

The striking effect of a single 10 mg. dose of 3-methylcholanthrene on the urinary excretion of ascorbic acid is shown in Figure 6 (114). By six days after administration of the hydrocarbon, the rate of ascorbic acid excretion was 50 to 75 times greater than the control value, and, in fact, during the 19-day period of the experiment about 140 mg. of the vitamin

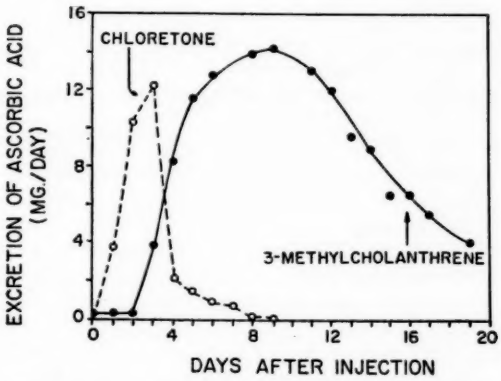


FIG. 6. Stimulation of L-ascorbic acid excretion by 3-methylcholanthrene and chlorobutanol (Chloretone).

was recovered in the urine. For comparison, the effect of a single 40-mg. dose of chlorobutanol is also shown. It will be noted that chlorobutanol exerts an immediate effect on ascorbic acid excretion which reaches a peak by the third day and falls to a low value by the fifth day. With 3-methylcholanthrene, no effect was observed for two days, but after this period the excretion values remained markedly elevated for at least 18 days. No explanation can be offered at present for the unusually prolonged effect of 3-methylcholanthrene on ascorbic acid synthesis.

Various drugs have been reported (112, 114) to exert little or no effect on ascorbic acid excretion in rats: urethane, acetanilide, phenacetin, *p*-aminophenol, phenol, sulfanilamide, codeine, quinine, salicylates, borneol, α-naphthol, phenolphthalein, ethyl biscoumacetate (Tromexan), reserpine, chlorpromazine, chlorzoxazone, carbon tetrachloride, sodium bromide, nicotinamide, and keto-oxyphenbutazone. Interestingly enough, Minesita *et al.* (118) observed that the administration to rats of the alkaloid lycorine inhibited ascorbic acid synthesis and decreased ascorbic acid content of various organs, producing a condition resembling scurvy. Other studies by Conney *et al.* (119) showed that lycorine administration to rats antagonized the stimulatory effect of chlorobutanol on ascorbic acid synthesis.

Available evidence indicates that the drug-induced synthesis of ascorbic acid results from increased metabolism of glucose through the glucuronic acid pathway (Fig. 7). The existence in animals of this route of glucose metabolism has been revealed largely by studies on the biosynthesis of L-ascorbic acid and L-xylulose. The reactions of this pathway have been reviewed recently by Touster (120), Burns (121), Strominger (122), and Burns & Conney (123). According to this scheme, D-glucose is oxidized to D-glucuronic acid, which undergoes reduction to L-gulonic acid; the latter

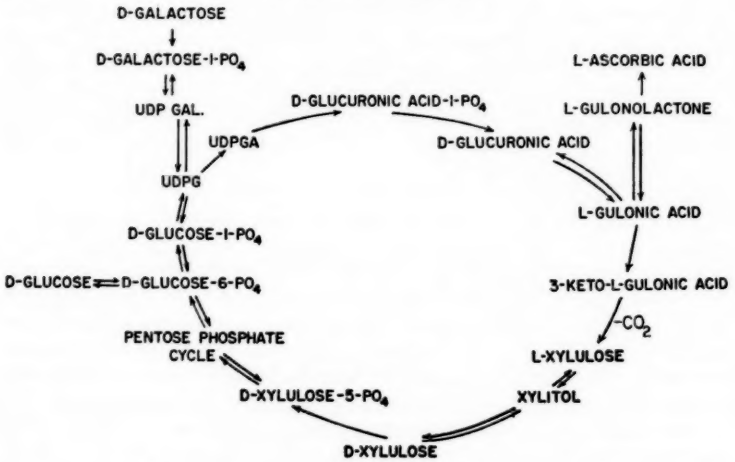


FIG. 7. The glucuronic acid pathway of glucose metabolism.

acid then serves as the precursor of either L-ascorbic acid or L-xylulose. Administration of barbital or chlorobutanol to rats stimulates the conversion of D-glucose-1-C^{14} or D-galactose-1-C^{14} to labeled D-glucuronic, L-gulonic, and L-ascorbic acids (114, 124, 125). Administration of barbital to guinea pigs, a species which cannot make ascorbic acid, leads to the increased urinary excretion of D-glucuronic acid (124). Martin *et al.* (126) observed no detectable conversion of D-galactose-1-C^{14} to ascorbic acid in guinea pigs treated with 3-methylcholanthrene. It should be noted that the guinea pig, as well as man and monkey, lacks the ability to convert L-gulonolactone to L-ascorbic acid (121).

Further evidence for the effect of drugs on the glucuronic acid pathway came from earlier studies by Enkelwitz & Lasker (127). They showed that aminopyrine and antipyrine, which stimulate the synthesis of ascorbic acid in the rat, also markedly elevate the urinary excretion of L-xylulose in human subjects with essential pentosuria. This observation can be explained in terms of the scheme in Figure 7, since administration of these drugs would be expected to increase the formation of L-xylulose from D-glucose. The pentosuric subject is not able to metabolize L-xylulose (120), and thus the pentose would be excreted in the urine.

The mechanism by which drugs stimulate the formation of ascorbic acid is not known. The possibility that a renal mechanism is involved has been ruled out since chlorobutanol induces the synthesis of the vitamin in nephrectomized rats (114). The available evidence (111, 114, 121) indicates that drugs such as chlorobutanol and barbital increase ascorbic acid bio-

synthesis by stimulating the formation of free D-glucuronic acid. Increased synthesis of labeled free D-glucuronic acid from D-galactose-1-C^{14} has been observed by Evans *et al.* (125) in liver homogenates of rats pretreated with chlorobutanol, but the specific enzymatic steps involved have not yet been defined.

The formation of a glucuronide by the drug is not required since barbital, one of the most potent drugs stimulating the synthesis of ascorbic acid, is recovered unchanged in the urine (124). It should be noted that borneol and aniline, potent in forming glucuronides, have no effect on the synthesis of ascorbic acid or its precursor, L-gulonic acid. Aniline was shown by Smith & Williams (128) and by Axelrod *et al.* (129) to cause appreciable excretion of free D-glucuronic acid resulting from the breakdown of a labile aniline N-glucuronide.

Ganguli *et al.* (130) reported that the administration to rats of adenosine triphosphate, malic acid, or thyroxine suppresses the chlorobutanol-stimulated synthesis of ascorbic acid. They also showed the administration of chlorobutanol elevates the inorganic phosphorus level in liver while the levels of several hexose and triose phosphates are diminished. Straumfjord & West (131) reported that the stimulation of ascorbic acid formation by chlorobutanol is not impaired in the alloxan-diabetic rat. The relevance of these findings to the effect of drugs on ascorbic acid synthesis is not clear.

Many reports have appeared in the literature showing that a variety of drugs depress the level of ascorbic acid in the adrenal glands. However, there appears to be no obvious relationship between this particular drug effect and the effect of drugs on ascorbic acid synthesis. Administration of barbital or chlorobutanol to adrenalectomized rats produces about the same increase in ascorbic acid excretion as that observed in normal rats (124). Possible hormonal control over this phenomenon is suggested from results showing that the effect of chlorobutanol and barbital on ascorbic acid excretion is less in hypophysectomized rats than in normal rats (124).

Whatever mechanism is proposed for the stimulatory effect of drugs on ascorbic acid synthesis must take into consideration that the amount of glucose metabolized through the glucuronic acid pathway is greater than the amount of drug administered; in fact the amounts of free D-glucuronic, L-gulonic, and L-ascorbic acids excreted in urine of drug-treated rats are minimum values for the total amount of these acids formed since they are in turn extensively metabolized (116, 117, 132).

The enhanced formation of ascorbic acid through the glucuronic acid pathway may represent an adaptive response of the body to foreign compounds. Reports have recently appeared (133) indicating that the administration of D-glucuronolactone reduces the toxicity of various foreign compounds in animals by an unknown mechanism. It is of considerable interest that those drugs which are potent in stimulating the synthesis of ascorbic acid, such as phenobarbital, barbital, aminopyrine, and the previously men-

tioned carcinogenic hydrocarbons, have been shown by Conney & Burns (134) and Conney *et al.* (135) to increase the activity of liver microsomal enzymes which metabolize various foreign compounds. This observation suggests a relationship between the stimulatory effect of drugs on ascorbic acid synthesis and on their ability to increase the activity of drug metabolizing enzymes in liver microsomes.

Ascorbic acid deficiency may also influence the pharmacological effect of drugs. For example, ascorbic acid-deficient guinea pigs are more sensitive to the action of theophylline (136), ether (137), pentobarbital (138), procaine (139), and zoxazolamine (119). It is possible that ascorbic acid may exert this effect, at least in part, by influencing drug metabolism. Udenfriend *et al.* (140), Brodie *et al.* (141), and Dalgliesch (142) have shown that a model system consisting of ascorbic acid, ferrous ion, ethylenediaminetetraacetic acid, and oxygen can catalyze the hydroxylation of acetanilide, antipyrine, aniline, anthranilic acid, and kynurenine to yield products identical with those formed in the body. Axelrod *et al.* (136) further reported that the rate of hydroxylation of several of these compounds is markedly reduced in guinea pigs depleted of ascorbic acid and that on repletion of the animals with ascorbic acid the rate of hydroxylation is restored to normal. Although these results suggest a role for the vitamin in the metabolism of drugs, attempts to define such a function at the enzyme level have not been successful. Conney *et al.* (119) recently reviewed the various metabolic interactions between ascorbic acid and drugs.

Stimulatory Effect of Drugs on Drug-Metabolizing Enzymes

Many drugs are metabolized by enzymes in liver microsomes which require reduced triphosphopyridine nucleotide and oxygen. These enzyme systems have been reviewed recently by Brodie *et al.* (143) and by Axelrod (144). Several laboratories reported that the administration of various foreign compounds markedly increases the activity of these drug-metabolizing enzymes in liver microsomes. Conney *et al.* (145, 146) observed that pretreatment of rats with polycyclic hydrocarbons such as 3,4-benzpyrene, 3-methylcholanthrene, and 1,2,5,6-dibenzanthracene greatly increases the activity of enzyme systems in liver microsomes which N-demethylate 3-methyl-4-monomethylaminoazobenzene, hydroxylate 3,4-benzpyrene, and reduce the azo linkage of 4-dimethylaminoazobenzene. Subsequent studies by Conney and co-workers (134, 135, 147, 148) showed that pretreatment of rats with a variety of compounds such as phenobarbital, barbital, aminopyrine, phenylbutazone, orphenadrine, chlorcyclizine, and 3,4-benzpyrene causes a marked increase in the activity of the enzymes in liver microsomes which metabolize hexobarbital, pentobarbital, aminopyrine, phenylbutazone, 3-methyl-4-monomethylaminoazobenzene, 3,4-benzpyrene, and zoxazolamine. The ability of phenobarbital to stimulate the activity of liver microsomes

to oxidize hexobarbital and to N-demethylate monomethylaminopyrine was observed by Remmer (149, 150) in rats, mice, and rabbits. Inscoe & Axelrod (151) reported that the activity of liver microsomes to form *o*-aminophenol glucuronide is increased by administration of 3,4-benzpyrene and 3-methylcholanthrene. Cramer *et al.* (152) showed that the administration of 3-methylcholanthrene causes a marked increase in the ability of liver microsomes to hydroxylate the carcinogen 2-acetylaminofluorene.

Administration of certain drugs can actually enhance the ability of liver microsomes to metabolize the same or a closely related compound (135). Thus, the administration of phenylbutazone, aminopyrine, 3,4-benzpyrene, or phenobarbital will increase, respectively, the ability of rat liver microsomes to metabolize phenylbutazone, aminopyrine, 3,4-benzpyrene, or hexobarbital.

Pretreatment of rats with certain drugs can appreciably influence the pharmacological activity of other drugs as shown by the following examples:

(*a*) Pretreatment of rats with drugs that stimulate the zoxazolamine-metabolizing enzyme system shortens the duration of action of zoxazolamine (135, 153). Since this drug is hydroxylated to a pharmacologically inactive metabolite (154), any increase in the activity of the zoxazolamine-metabolizing enzyme system in liver would be expected to shorten the duration of a paralytic dose of zoxazolamine. Indeed, this was found to be the case. Pretreatment of rats with phenylbutazone, aminopyrine, barbital, orphenedrine, phenobarbital, or 3,4-benzpyrene, all of which increase the activity of the zoxazolamine-metabolizing system in the liver microsomes, causes a concomitant decrease in the duration of zoxazolamine paralysis in the intact animal. For instance, the duration of zoxazolamine paralysis in control rats averages 730 minutes, in phenobarbital-treated rats 102 minutes, and in 3,4-benzpyrene-treated rats only 17 minutes.

(*b*) Pretreatment of rats with phenobarbital, phenylbutazone, chlorcyclizine, or orphenadrine noticeably increases the activity of the liver microsomes to metabolize hexobarbital, and this effect is paralleled by a shortened duration of action of hexobarbital (135). For example, control rats given hexobarbital sleep for an average of 216 minutes, while phenobarbital pretreated rats given hexobarbital sleep for an average of 11 minutes. The ability of phenobarbital to stimulate markedly the metabolism of hexobarbital has led Remmer (149, 150) and Conney and co-workers (135) to suggest that tolerance to barbiturates may result, at least in part, from an accelerated rate of metabolism of the barbiturate to pharmacologically inactive metabolites. Although pretreatment of rats with chlorcyclizine, orphenadrine, or phenobarbital shortens the duration of action of hexobarbital by accelerating its metabolism, pretreatment of rats with these drugs does not shorten the duration of action of barbital, a drug which is not metabolized in the rat (148). It should be noted that the stimulatory effect of drugs

upon drug metabolism furnishes an explanation for the recent findings of Thompson *et al.* (155) that rats fed on a diet containing chlorcyclizine develop resistance to the hypnotic action of pentobarbital.

(*c*) Murphy & DuBois (156) reported that the administration of 3-methylcholanthrene, 3,4-benzpyrene, or 1,2,5,6-dibenzanthracene increases markedly the activity of the enzyme system in liver microsomes which catalyzes the conversion of the dimethoxy ester of benzotriazine dithiophosphoric acid (guthion, BDB) and ethyl *p*-nitrophenylthiobenzenephosphonate (EPN) to their corresponding oxygen analogues which are active anticholinesterase agents. In contrast to the above cited examples where the hydrocarbons increase the conversion of drugs to inactive metabolites, the hydrocarbons in this case increase the conversion of the organic thiophosphates to active metabolites. For example, the inhibition of cholinesterase caused by the intraperitoneal administration of 2 mg./kg. of guthion was increased by approximately 50 per cent 48 hours after the injection of 3-methylcholanthrene.

Several lines of evidence (135, 145, 146) suggest that compounds such as 3,4-benzpyrene and phenobarbital increase the activities of drug-metabolizing enzymes by inducing enzyme synthesis. Administration to rats of the amino acid antagonist, DL-ethionine, completely prevents the phenobarbital- or hydrocarbon-induced increases in the activity of the azo dye demethylase or zoxazolamine metabolizing system, whereas the administration of DL-methionine nullifies the inhibitory action of DL-ethionine. Administration of phenobarbital, 3-methylcholanthrene, and 3,4-benzpyrene also stimulates liver growth. Livers from drug-treated rats are usually 20 to 30 per cent heavier than the livers from controls, and the amount of microsomal or total liver protein is increased proportionately.

Administration of drugs which stimulate the drug-metabolizing enzymes in liver microsomes can also exert other biochemical responses in animals. Pretreatment of rats with chlorcyclizine or phenobarbital markedly stimulates the activity of the liver-microsome system that oxidizes reduced triphosphopyridine nucleotide (148). Methylcholanthrene-treated rats were reported by Glenn *et al.* (157) to have increased hepatic activity for the metabolism of hydrocortisone. Jondorf *et al.* (158) found that liver tryptophan peroxidase activity was markedly increased and that serum alkaline phosphatase was substantially decreased following administration of 3-methylcholanthrene. Conney & Burns (134) and Burns *et al.* (114) reported that those drugs which stimulate the activity of drug-metabolizing enzymes in rats also stimulate ascorbic acid synthesis.

Effect of Drugs on Cholesterol Metabolism

During the past several years considerable efforts have been made to find compounds that inhibit cholesterol synthesis. These studies have been motivated by the hypothesis that hypercholesterolemia is one of the predis-

posing factors in the genesis of atherosclerosis. One such compound is triparanol (MER-29) which has been introduced recently into clinical practice. Blohm & McKenzie (159) were the first to report on the striking inhibition of cholesterol synthesis brought about by the administration of triparanol to rats. They found that the drug reduces the incorporation of acetate-1-C^{14} into cholesterol of liver and intestine of intact rats. In a subsequent study, Blohm *et al.* (160) showed that triparanol decreases the levels of cholesterol in the following tissues and fluids of the rat: blood plasma, erythrocytes, liver, adrenal, lung, aorta, skeletal muscle, and bile. In a recent conference triparanol was reported (161) to lower plasma cholesterol in a majority of patients.

Avigan *et al.* (162) have presented evidence for the mechanism of action of triparanol. They showed that the major block in cholesterol synthesis caused by the drug is at the last step—the conversion of 24-dehydrocholesterol (desmosterol) to cholesterol. The accumulation of significant amounts of the precursor sterol not only in liver but also in the serum of drug-treated animals and patients has been reported by Steinberg & Avigan (163) and by Blohm *et al.* (164).

Other compounds have also been studied for their ability to lower cholesterol levels: phenylethyl acetate (163, 165), Δ^4-cholestenone (163), sodium dextro-thyroxine (166), 3,5-diiodothyroacetic acid and 3,5-diiodothyroformic acid (167), and benzmalacene (168). These compounds appear to have modes of action different from that of triparanol, and their clinical value remains to be established.

Effect of Drugs on Uric Acid Biosynthesis and Excretion

Gout is a disease characterized by overproduction of uric acid leading to deposits of this substance in various tissues, especially in the joints. Drugs such as probenecid, certain phenylbutazone analogues, and zoxazolamine which increase the urinary excretion of uric acid are of considerable value in the treatment of gout. Although it is fairly well established that these drugs act by blocking the reabsorption of uric acid through the renal tubular cells, the structural features required in a drug for this activity are still not clear. Burns *et al.* (169) have reported a relationship between the chemical structure of phenylbutazone and its uricosuric activity. They found that modifications in phenylbutazone which result in increased acidity of the molecule are associated with enhanced uricosuric activity, whereas changes which decrease the acidity of the drug are accompanied by loss of this activity. The potent uricosuric activity of the highly acidic phenylbutazone analogues (pK_a 2.0 to 3.0) indicates that these drugs act in the ionic form to block the reabsorption of uric acid by the renal tubular cells, thus furnishing a possible clue to the still obscure nature of the transport mechanisms involved. The demonstration that uricosuric activity in the phenylbutazone series is related to the pK_a has proved of considerable aid

in searching for new uricosuric drugs. One such drug, sulfinpyrazone, has been found to be useful clinically as a potent uricosuric agent in the treatment of gout, and others are now under study (170).

Another approach to the treatment of gout is the use of drugs which would suppress the synthesis of uric acid. Grayzel *et al.* (171) have reported recently that 6-diazo-5-oxo-L-norleucine inhibits significantly the synthesis of uric acid in gouty patients. This drug, a structural analogue of glutamine, presumably prevents the synthesis of inosinic acid by blocking the utilization of glutamine in the enzymatic conversion of α-N-formylglycinamide ribotide to formylglycinamidine ribotide. However, the high incidence of side effects following administration of the compound prevents its practical use in the treatment of gout. Studies with this drug, nevertheless, represent a new approach to gout therapy; perhaps, safer pharmacological agents of this type will be forthcoming. Interestingly enough, Seegmiller *et al.* (172) have recently reported that 2-ethylamino-1,3,4-thiadiazole actually stimulates the synthesis of uric acid. It is possible that further studies with this compound will be of value in determining the factors which normally operate to control purine biosynthesis in man.

Primaquine-Induced Hemolytic Anemia

Administration of the antimalarial drug, primaquine, and certain other 8-aminoquinoline derivatives induces an acute hemolytic anemia in some individuals (173, 174). The incidence of this primaquine sensitivity is much greater in Negroes than in Caucasians, and the condition is apparently genetically determined (175). A similar hemolytic crisis can be induced by ingestion of fava beans, naphthalene, acetanilide, phenylhydrazine, and nitrofurantoin.

Studies by Carson *et al.* (176) indicate that primaquine-induced hemolytic anemia results from a biochemical defect which is characterized by markedly reduced activity of glucose-6-phosphate dehydrogenase in red blood cells. This enzyme requires triphosphopyridine nucleotide as a cofactor and catalyzes the first step in the metabolism of glucose through the hexose monophosphate shunt. An alteration in glutathione metabolism also occurs in the sensitized erythrocytes; this presumably results from the impaired reduction of triphosphopyridine nucleotide caused by the defect in glucose-6-phosphate dehydrogenase. Certain of the reactions of erythrocyte metabolism which are affected, either directly or indirectly, by the biochemical abnormalities in red cells of sensitive individuals are illustrated in Figure 8. Alterations in glutathione metabolism and deficiencies in glucose-6-phosphate dehydrogenase have also been reported (174) in erythrocytes of subjects sensitive to the fava bean and naphthalene.

Primaquine-sensitive hemolytic anemia represents an excellent example of a drug toxicity that can be attributed to a defect in an enzyme system.

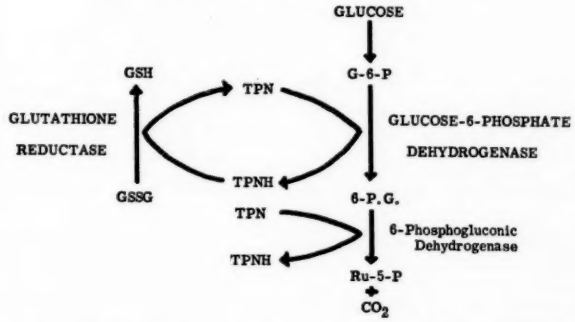


FIG. 8. Schematic representation of certain reactions of erythrocyte metabolism which are affected by the defect present in red cells of subjects with primaquine-sensitive hemolytic anemia. Abbreviations employed: G-6-P, glucose-6-phosphate; 6-P.G., 6-phosphogluconic acid; Ru-5-P, ribulose-5-phosphate; TPN, triphosphopyridine nucleotide; TPNH, reduced triophosphopyridine nucleotide; GSSH, oxidized glutathione; GSH, reduced glutathione. This scheme is taken from the review of Marks & Gross (174).

Furthermore, the importance of genetic control in a drug sensitivity is well illustrated by studies on this condition. The possibility that other drug sensitivities may also result from genetically transmitted enzyme deficiencies might be fruitful to explore.

CONCLUDING REMARKS

As evidenced by even the limited number of topics reviewed in this chapter, studies of the biochemical effects induced by drugs can contribute greatly to a better understanding of the possible mechanisms of action of the drugs. More important, perhaps, is the insight that a knowledge of drug effects can give us into normal metabolic processes. This extra dividend is perhaps the most significant factor in making the relatively new field of biochemical pharmacology increasingly important.

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RECENT LABORATORY STUDIES AND CLINICAL OBSERVATIONS ON HYPERSENSITIVITY TO DRUGS AND USE OF DRUGS IN ALLERGY

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The classification of reactions to drugs as allergic or clearly nonallergic (the latter consisting of hyperreactions and meta-reactions) has been previously reviewed (17) with reference to the definition of allergy itself. In the previous review it was suggested that the term hypersensitivity be used for reactions that could not yet be clearly classified as either allergic or nonallergic in nature. The present review is concerned chiefly with clinical observations and laboratory studies published during the period June, 1959 to May, 1960 and will be restricted to examples of drug allergy and drug hypersensitivity, as defined above. Clear-cut hyperreactions and poisoning by gross overdosage will be omitted. The authors must point out that their terminology has no particular intrinsic merit over that used by other writers. If the reader wishes further details of the terms briefly described above, he can, by referring to the published definition of the terms (17), learn what types of reactions are and are not likely to be considered in this review. The basis for deciding that a given reaction probably has no allergic basis has been previously discussed (16, 17, 18), but decisions will continue to be difficult. Many of the hypersensitivities considered in this review may eventually prove not to depend on the presence of antibodies, but this fact does not make them any less interesting or important.

The recent development of techniques permitting the demonstration of circulating antibody in the serum of certain patients allergic to penicillin suggests that one may some day hope to demonstrate antibodies in many other reactions that are already, on clinical grounds, considered to be allergic in nature. If this should come about, we may then be justified in concluding that failure to demonstrate antibody in other, more dubious situations strongly suggests that no antibody exists. At present such a conclusion would be rash, and even in the future one would wish to search for antibodies not only in the serum but elsewhere. The work of Ley *et al.* (71) with "penicillinized" erythrocytes has been followed by a recent interesting study in which Epp (31) demonstrated circulating antibody against penicillin in nine of 20 persons stated to be allergic to penicillin. Epp used bisdiazotized benzidine to couple penicillin with erythrocytes and detected the

presence of antibody by hemagglutination. Furthermore, specific inhibition by the antigen was demonstrated. Cross-reactions between different penicillins suggested that the penicillin molecule per se not the side chain, was the antigen. Unfortunately, the report does not indicate whether only those patients with reactions such as anaphylaxis or urticaria had demonstrable antibody in their serum or whether patients with, for example, dermatitis had such antibody also. By the Prausnitz-Kustner technique antibodies were demonstrated in two of seven post-mortem sera of patients who had died of penicillin anaphylaxis (80).

Before hyperreactions and intoxications from overdosage are dismissed, it would perhaps be useful to describe a few instances reported during the period covered by this review in order that the reader may decide for himself whether he considers the classification and restrictions valid. Evidence of extrapyramidal disturbances produced by prochlorperazine (Compazine) in eight patients (104) probably indicates hyperreaction even though one of the eight had a past history of allergy to a sulfonamide, one patient was known to be allergic to peanuts, and not all the patients had received large doses. The known effects of phenothiazines on the central nervous system are such that the changes observed in these patients probably represent merely an exaggeration of a fundamental pharmacological effect of the drug. The reported patients were presumably individuals who would be among the most responsive if a dose-response curve for this particular effect were carried out in a large population. As another example, the series of 16 patients who received large doses of ristocetin (the definition of a large dose in this series being more than 50 mg./kg. per day or the use of a solution of more than one mg./ml. in concentration) probably did not all have allergic reactions to the drug (42). Among the 16 patients, 11 had hematologic complications; decrease in platelets, leucocytes, and erythrocytes were all observed. However, the author of the above report found in rabbit experiments and *in vitro* studies that direct destruction of platelets could occur when high concentrations of the drug were used. The reaction was reversed in man and animals when the dosage was reduced, but skin rash and fever were also reported in several patients. Therefore, even though the concentration of ristocetin achieved in the patients was apparently sufficient to cause a direct effect on platelets, it would still be unwise to say that allergic reactions to this drug do not occur (see also the discussion of phenacetin, furaltadone, etc., below). The leukopenia, anemia, and gastrointestinal reaction produced by desacetyl methyl colchicine (27) were also most probably a result of overdosage in the instance reported, although the occurrence of a maculopapular rash in the patient should be noted.

Anaphylaxis

Acute, severe systemic reactions may be produced by a variety of drugs, including chemotherapeutic agents, horomones, local anesthetics, x-ray con-

trast media, sclerosing agents, and, occasionally, other injected, ingested, or inhaled drugs. Antigenic substances deliberately used in skin testing or hyposensitization are also obviously potentially dangerous (119). However, it appears likely that sclerosing agents, when inadvertently released in appreciable amounts into the general circulation, may cause direct intoxication needing no antibody for its mediation. The frequency with which reactions to x-ray contrast media occur immediately after the initial injection of the agent has been noted by several observers. Although most types of reaction usually reported to occur after a clearly recognizable period of sensitization may occasionally occur on first administration to a person with no known previous exposure [as in 27 of 216 cases of reactions to penicillin (73)], the reactions that occur during intravenous urography and other procedures using intravenous injections of similar compounds have usually been reported after initial exposure. A recent review (50) records 31 deaths in more than three million urograms.

Apropos of the occurrence of clearly allergic reactions after first known exposure to a drug, one often speculates that a previous, unknown exposure sensitized the patient. This speculation is consistent with Ljaljevic's (73) finding that penicillin, the commonest drug allergen in his series, was also the commonest cause of allergic reactions occurring promptly after first known exposure, for one would logically expect this widely used and frequently antigenic drug to be a common source of unknown or forgotten sensitizing exposure. Penicillin in foods may play a role here (see below) but forgotten exposure from previous medical treatment seems highly possible.

Local anesthetics are known to produce clearly allergic reactions, e.g., contact dermatitis, and allergic reactions may account for some of the deaths following use of local anesthetics. For example, a patient who developed respiratory distress and died 20 minutes after taking a throat lozenge containing benzocaine and tyrothricin was shown at autopsy to have edema of the larynx (54). Although tyrothricin cannot be exculpated with absolute certainty, the known propensity of local anesthetics to cause allergic reactions makes the benzocaine a much more likely offender. However, the known effects of local anesthetics on the central nervous system and on the myocardium make convulsions or sudden severe hypotension, respectively, likely whenever a large "bolus" of injected local anesthetic accidentally reaches the brain or myocardium. Therefore, many of the severe and even fatal reactions that occur after topical use of local anesthetics, as in dentistry, may well be related to vagaries of absorption rather than to allergy (2). Incidentally, the decision that some new local anesthetic is to be labeled by fiat and decree "not a 'caine' drug" will probably not confer any magical lack of toxicity on the drug.

It is not surprising to find that various proteins injected for therapeutic purposes may induce severe systemic reactions; gamma globulin (5), chymotrypsin (72), and other preparations of blood proteins (10) have

caused severe reactions. These reactions should not necessarily be expected always to follow the pattern of classic anaphylaxis, since direct effects from certain proteins, particularly enzymes, might also be expected. However, in a patient who developed hypotension, angioedema, and fever after intravenous injection of human fibrinolysin, immunologic studies suggested the presence of antibodies against fibrinolysin and against streptokinase activator (10).

Recent reports of severe systemic reactions from drugs include an instance of facial edema, urticaria, and shock after intravenous administration of succinyl choline, with recurrence of the skin reaction on readministration (66) and an instance of dyspnea, chest pain, urticaria, and angioedema occurring 10 minutes after the first intravenous injection of vancomycin to a patient with a previous history of allergy to sulfonamides (100). The latter patient is stated to have subsequently developed an allergic reaction to novobiocin. Although anaphylaxis to penicillin is well known, a recent report of electrocardiographic changes observed during such a reaction (7) is of interest. Auricular fibrillation, conduction defects, and evidence of myocardial injury were noted. The author of the report suggests that the heart itself may actually participate in the anaphylactic reaction. However, the possibility of cardiac changes secondary to other effects of anaphylaxis, such as anoxia, is difficult to rule out.

The data reviewed by Seevers (106) suggest an incidence of one to 10 deaths from anaphylaxis per 10 million injections of penicillin. It has been estimated that about 10 per cent of the cases of penicillin-induced anaphylaxis result in death (25).

Serum Sickness Syndrome

Neuritis has previously been reported to be an occasional accompaniment of the serum sickness syndrome. Farme (35) has reported pachymeningitis as an apparent sequel developing slowly after a serum sickness reaction probably due to penicillin. The patient recovered after craniotomy and cortico-steroid therapy.

Angioedema and Urticaria

Angioedema and urticaria have been described as part of the systemic reaction in certain instances already noted in the section on anaphylaxis (10, 54, 66, 100). Death from angioedema of the larnyx following administration of sulfobromophthalein has been reported (111). A report of angioedema of the gastric mucosa, mimicking carcinoma, is of interest (11). In addition, erythromycin (93) and griseofulvin (46) have been presumptive causes of urticaria in recent reports; the reaction to the latter drug also included angioedema. Urticaria was again reported as the commonest form of reaction to penicillin (73).

Dermatitis

Epstein & Kligman (33) showed that contact dermatitis requires the participation of intact vessels and depends upon the passage of cells rather than fluid from the lumen of the vessels into the extravascular space.

Exfoliative dermatitis has in the past been known to be associated with simultaneous damage in internal organs, e.g., the liver. In the past year reports of exfoliative dermatitis occurring after administration of penicillin (116) and phenindione (14) have noted the occurrence of simultaneous renal damage. The patient with renal insufficiency after penicillin had improvement in renal function after administration of penicillinase. The patient reacting to phenindione had jaundice as well as the presence of protein, casts, and white and red blood cells in the urine. Although the hematuria following administration of phenindione may have simply been attributable to the anticoagulant effect of the drug, the other urinary findings suggest more extensive renal injury.

A report of contact dermatitis in a woman who had been working with a new phenothiazine derivative (47) serves to remind us of two points previously established in drug allergy. First, the onset of contact dermatitis in workers exposed to new drugs in the course of their development may be an omen of subsequent sensitizing capacity of the drug in the clinic. This has previously been noted with drugs such as penicillin. Second, patch tests in the above patient showed cross-reactions with those phenothiazines which, like the compound that originally sensitized her, contained a chlorine in the 2 position on the phenothiazine ring. Although the results suggested that the nature of other substituents on the phenothiazine ring was less significant, the compound 2-chlorophenothiazine was too insoluble to allow satisfactory testing in this study, and the role of other substituents, in addition to chlorine, could not be entirely dismissed. Phenothiazine compounds without a chlorine atom in this position did not cause cross-reactions. The correlation of sensitization with the structure of one portion of a molecule has previously been noted with other drugs, e.g., local anesthetics.

Twenty-eight cases of contact dermatitis caused by neomycin were reported (95). Cross-reactions between neomycin and streptomycin may occur (41). Exfoliative dermatitis (77) and erythema multiforme with the Stevens-Johnson syndrome (43) were reported after administration of sulfamethoxypyridazine (Kynex). The role of this sulfonamide in causing the exfoliative dermatitis was confirmed by the fact that a challenging readministration of the drug subsequently caused a recurrence of the reaction in a lesser form. Although this is of considerable scientific interest, one wonders whether the challenge is wise in a patient who has undergone such a severe reaction. A fatal septicemia has been recorded (102) as the final complication of a severe skin reaction to this drug. Among the more recently introduced drugs that can apparently produce skin and mucosal reac-

tions is chlorpropamide (59, 101, 112). However, the incidence of sensitization to this drug is difficult to determine, because the patients who are taking it are almost invariably diabetic and the development of itching and skin eruptions in diabetic patients may be due to other causes. In a report of several instances of contact dermatitis (sometimes with subsequent persistent changes in pigmentation) caused by local application of strong concentrations of mono-benzylether of hydroquinone, Dorsey (28) has estimated the incidence of contact dermatitis from this ointment as 13 per cent.

Photosensitivity after administration of a drug is a problem that is presumably separate from the one under consideration in this review. Therefore, photosensitivity will be mentioned here only to record that the list of drugs capable of provoking photosensitivity has been expanded by the inclusion of some recently developed drugs, including chlorothiazide and hydrochlorothiazide (52, 53, 88) and demethylchlortetracycline (Declomycin) (34, 83). Promethazine (Phenergan) has continued to cause dermatitis; a report (32) of dermatitis appearing at the site of local application after exposure to light (photocontact dermatitis) demonstrated this phenothiazine to be the offender. Biopsy showed vesicles and eosinophilia. A useful discussion of dermatologic reactions to drugs is found in Fromer's (41) review.

Depression of the Blood and Blood-Forming Organs

Reviewing records of 66 patients who died from agranulocytosis in Denmark during the period 1951 to 1957, Mosbech & Riis (84) found 25 cases attributable to drugs. The physician who once sees a patient with one or several of the lines of formed elements of the blood wiped out by a drug reaction is not likely to forget the experience, particularly if he sees a patient die from this complication and finds, on reviewing the history, that the patient had received the drug for a relatively minor indication. A previous brief review (18) noted the evidence that certain instances of depression of white blood cells or platelets are very probably mediated by antibodies, whereas certain drug-induced anemias have been clearly shown to be associated with an enzymatic defect. In the aforementioned review it was pointed out that laboratory and clinical studies had made possible a prediction that certain drug-induced hemolytic anemias would be found to be nonallergic in nature, a fact subsequently demonstrated. However, it is obvious that drugs capable of causing a reaction such as hemolytic anemia through a nonallergic mechanism may still cause other disturbances that probably depend upon the presence of antibody. This situation has, in fact, been reported. Thus, furaltadone has been reported (21) to cause a maculopapular and urticarial skin eruption. Thrombocytopenia and leukopenia have also occurred in patients receiving furaltadone (21). Even more striking is the report that phenacetin and *p*-aminosalicylic acid may cause hemolytic anemia in some patients by a mechanism requiring the presence of

antibodies (74). Thus, the drugs apparently cause the same reaction by different mechanisms in different patients.

Circulating substances causing agglutination or lysis of platelets have been found in many instances of drug-induced thrombocytopenia. Bishop, Spencer & Bethell (9) have pointed out that search for antibodies *in vitro* now has clinical value as a preliminary test and sometimes obviates the need to consider the riskier *in vivo* challenge. Circulating leuko-agglutinins have occasionally been demonstrated in leukopenia. Circulating antibodies against certain other diseased tissues, e.g., heart, are currently thought not to be causally related to the disease of the antigenic organ (19, 85). Nevertheless, the antibodies against formed elements of the blood have been shown to be directly destructive of the blood cells in certain instances, and the presence of antibodies against white cells or platelets is consistent with certain other facts pointing to the allergic nature of such drug reactions. These considerations have been previously reviewed (17, 18).

The demonstration that certain instances of leukopenia or thrombocytopenia are apparently mediated by antibodies is still a far cry from implicating antibodies in all such depressions. Several possible objections must be considered. First, in many instances of leukopenia or thrombocytopenia a search for antibodies has failed to reveal any. For example, in a recent report (20) of thrombocytopenia caused by diethylstilbestrol and confirmed by repeated recurrences on readministration, no antibodies were found. In this instance there was also a moderate leukopenia of 2000 to 5000. The authors point out that estrogens have previously been reported to cause marrow aplasia with hypoplastic anemia and thrombocytopenia. We have the impression that antibodies have been demonstrated most often in instances of "pure" thrombocytopenia, rather than thrombocytopenia associated with depression of other formed elements of the blood. The estrogen-induced thrombocytopenia reported above did have one manifestation vaguely suggesting that an antibody may have existed in the skin, for the patient developed pruritus. There was no purpura.

Second, the frequency with which drugs containing the amino-benzene nucleus, or a closely related structure, cause depression of the formed elements of the blood leads one, rightly or wrongly, to recall the "bad reputation" of compounds related to para-aminophenol and phenylhydroxylamine with regard to effects upon the bone marrow. One may reasonably wonder whether the individual who suffers depression of the formed elements of the blood after receiving such drugs may not differ from the majority of individuals in that the unlucky individual metabolizes the drug in a different way rather than develops antibodies against it.

Third, the duration of "sensitization" should be noted. For example, administration of the phenothiazine drugs has been followed by agranulocytosis in numerous instances. Korst (69) reviewed 58 cases of agranulocytosis in patients who had received chlorpromazine and 11 cases in patients

who had received promazine. Pisciotta (91) reviewed 18 instances of agranulocytosis after administration of chlorpromazine. Whereas many allergic reactions to drugs occur most frequently during the second week of drug administration, agranulocytosis in patients receiving phenothiazines seems to occur most often during the fourth to sixth weeks (91) and sometimes even later, though usually within the first 15 weeks (69) after administration of the drug has begun. Agranulocytosis in patients receiving phenindione occurs most frequently after the third week but before the end of the third month (8, 108, 113). Furthermore, in a recent report of thrombocytopenia due to hydrochlorothiazide (45), not only was the initial reaction slow to appear after administration of the drug had commenced, but the recurrence that followed reinitiation of hydrochlorothiazide therapy apparently did not take place until the second course of administration had been carried out for more than two weeks. When thiouracil was widely used in the treatment of thyrotoxicosis, the onset of agranulocytosis was noted most commonly between the fourth and eighth weeks.

Obviously, compounds that tend to provoke agranulocytosis or thrombocytopenia only after a period of several weeks' administration will not attract attention as frequent causes of these reactions unless they are drugs likely to be administered for long periods of time. Tranquilizers, anticoagulants, diuretics, and antithyroid drugs, among others, meet this requirement. The treatment of tuberculosis offers another occasion to administer drugs for long periods. Thrombocytopenia caused by sodium para-aminosalicylate was first noted in one patient (49) after he had been receiving the drug for three years; readministration of the drug after recovery from thrombocytopenia led to a recurrence of the reaction in an hour. The report that 81 of 91 patients who suffered nonhematologic allergic reactions to various drugs during the initial course of systemic administration of the drug did so within the first 14 days (73) may, to some degree, simply reflect the fact that many of the drugs were not given in a prolonged course. [Thus, comparing a drug not likely to be given in a long course with one that is likely to be so given, only three of 55 penicillin reactions occurred after 14 days in patients receiving their first course of therapy, as contrasted to seven of 27 streptomycin reactions; the difference is significant ($P < 0.05$).] But the evidence still generally suggests that depression of the formed elements of the blood usually develops after a period of time that seems to be considerably longer than the sensitization period in the usual allergies. In the case of erythrocytes, one might consider the prolonged survival of circulating erythrocytes as a factor, but this explanation does not account for the prolonged "sensitization" before depression of platelets or leukocytes occurs. Furthermore, once the depression of platelets or leukocytes has started, it develops very rapidly in many instances.

One would like to reduce all depression of formed elements of the blood to one common effect of the offending drugs, but at present the only com-

mon effect that we can see even dimly is the possibility that both direct intoxication of the formed elements of the blood and depression mediated by antibodies depend, in the last analysis, upon the ability of certain compounds to react in some particular way with proteins of the formed elements. There seems little reason to doubt that depression of the formed elements, regardless of its cause, may involve both destruction of circulating elements and destruction of their precursors in the marrow or lymph nodes. It has previously been noted (18) that even when mediation by antibodies is assumed, one has no basis for knowing the exact pattern of the reaction until he can learn more about the exact location of antigen and antibody.

Hydrochlorothiazide (see above) was also implicated as a cause of several instances of thrombocytopenia in another report (87), and circulating factors causing platelet agglutination and lysis were shown in some cases in this report. Not all purpura attributable to drugs of the chlorothiazide group has been found to be thrombocytopenic. Like other drugs, chlorothiazide may precipitate purpura of the Henoch-Schonlein type (38). A patient who developed a leukopenia of 1300 (98 per cent lymphocytes) while receiving salicylazosulfapyridine (Azulfidine) had developed a skin rash on the fourth day after the beginning of drug therapy; the drug had, therefore, been discontinued for three days. Four days after the reinitiation of drug treatment the white blood count was 4350. The patient continued to take the drug for 23 days and at the end of this time was admitted to the hospital because of exhaustion. At this time the white blood count was found to be 1300. Twelve days after the administration of the drug had again been stopped the patient's serum contained a factor reacting with leukocytes in the presence of the offending drug (96). These cases are further indications of the fact that other, presumably allergic, reactions such as skin eruption may accompany drug-induced depression of the formed elements of the blood.

Regardless of the fact that a "sensitization" period of several weeks often elapses before development of drug-induced blood reactions in some patients, the occurrence of leukopenia (white blood count of 3500) in a patient who had been receiving tolbutamide for eight months (13) would still raise a considerable question about the causal role of the drug in such an instance, rare examples to the contrary notwithstanding. In fact, this patient was later shown to have acute leukemia. Leukemoid reactions may occur during the course of recovery from drug-induced leukopenia and have, of course, a more benign prognosis. A leukemoid reaction was reported during recovery from one of the aforementioned instances of phenindione-induced leukopenia (113).

Because patients with leukopenia are particularly susceptible to infection and because infection may also lead to depression of the bone marrow on occasion, one should not be surprised to find a history of exposure to chemotherapeutic agents in a certain number of patients who have leuko-

penia with or without depression of other formed elements of the blood. However, a history of exposure of chloramphenicol is found in a suspiciously high proportion of cases of aplastic anemia. In a recent study (105) of aplastic anemia, a modified tabulation of 607 cases of aplastic anemia originally reviewed by Welch, Lewis & Kerlan (122) showed that 26 had received only chloramphenicol, 66 had received chloramphenicol plus other drugs, and 32 had received sulfonamides. Chlortetracycline, oxytetracycline, barbiturates, anticonvulsants, and antihistaminic drugs had been given to 12, 11, 25, 17, and 8 patients respectively. Thirty-seven patients in this series had a history of exposure to antipyretics or related compounds. Aplastic anemia has followed chloramphenicol administration to patients suffering from mild infections not likely, per se, to cause depression of the bone marrow (22). The presence of the nitro- and amino-benzene group in the chloramphenicol and sulfonamide molecules, respectively, may, as previously noted, be a significant factor in their toxicity. When one considers the degree of increased risk of aplastic anemia resulting from chloramphenicol administration, he must in fairness admit that the increase in risk, though definite, seems slight. But physicians would be well advised to remember also that the increased risk, thought slight, seems definite. Although it would be difficult to deny the role of secondary infection in increasing the illness of patients with drug-induced agranulocytosis, the onset of the reaction itself seems to be accompanied by prompt systemic symptoms in many individuals, symptoms that are probably not entirely due to subsequent infection but are more probably associated with the drug reaction itself. Headache, fever, and general malaise were noted in some of the reports of phenindione-induced agranulocytosis mentioned above, and early systemic symptoms have been described in many previous reports of drug-induced agranulocytosis.

Other drugs implicated in recently reported instances of agranulocytosis are amodiaquine (67), chlorpropamide (112), and thenalidine (Sandostene) (1, 89). Sulfamethoxypyridazine has been implicated as a cause of thrombocytopenia (60), and, indeed, the latter drug has in other respects continued to carry on the bad name of the sulfonamide family in causing serious drug reactions. The over-all incidence of reactions to sulfamethoxypyridazine has been estimated (60) as about 6 per cent, this incidence being considerably less than that of reactions to sulfathiazole, slightly less than that of reactions to sulfadiazine, and somewhat more than that of reactions to sulfisoxazole (Gantrisin). The ability of certain sulfonamides to inhibit carbonic anhydrase was an important factor in the development of the diuretic drug, acetazoleamide. In acetazoleamide the substitution of a heterocyclic ring for the benzene ring of the sulfonamides has apparently not relieved this drug of the familial tendency to cause hematologic reactions. Fatal agranulocytosis from acetazoleamide was recorded in a report (57) in which instances of aplastic anemia and thrombocytopenia ascribed to

acetoazoleamide were also reviewed. Chloramphenicol and ristocetin have been mentioned above as causes of depression of formed elements of the blood; however, they are not the only antibiotics so implicated. In reviewing reactions to antibiotics, Seevers (106) noted that novobiocin and fumagillin have produced leukopenia in some patients.

The earlier hope that corticosteroids would decrease the mortality in patients with drug-induced agranulocytosis has, for the most part, met with disappointment. Since corticosteroid treatment of patients with normal numbers of leukocytes may increase the risk of infection, which may then sometimes secondarily depress the bone marrow, agranulocytosis may at times itself be an indirect complication of corticosteroid therapy. Such instances do not appear to be common, as compared with other complications of corticosteroid therapy. A possible example of this sequence of events is found in the case reported by Rosketh (97), though even here the history of previous irradiation of the patient makes interpretation difficult. Corticosteroids may well aid in the treatment of drug-induced thrombocytopenia. In the latter instance platelet transfusions can also be considered, but Bishop (9) has found these to have limited value in drug-induced thrombocytopenia.

In a particularly discouraging case of agranulocytosis following use of chlorpropamide (65), the white blood count, which was 2050 (6 per cent neutrophils) on the patient's admission, rose to 6000 (75 per cent neutrophils) after 10 days in which treatment consisted of corticosteroids, chemotherapy, and omission of the offending drug. Yet the marrow showed little evidence of recovery and cessation of corticosteroid therapy after four weeks was soon followed by death, the peripheral white blood count and neutrophil percentage having again fallen to low levels.

Hepatic Reactions

It now seems very likely that drugs may damage the liver by several distinct mechanisms. Although clinicians, pharmacologists, and pathologists have probably suspected this for a long time, at least two advances in important detail have been made relatively recently. First, the mechanism of "direct toxicity" of carbon tetrachloride has been studied (15) with results that bring us quite far from the attitude that was, in effect, our previous explanation of this toxicity, namely. "Of course it damages the liver—it's poison!" Second, the instances in which hypersensitivity appears to play a role in hepatic damage from drugs have also been separated into at least two categories, obstruction and hepatocellular damage, a dichotomy already well known in other types of liver disease. The drug-induced obstruction is, however, intrahepatic. Intrahepatic obstruction from drugs such as chlorpromazine is sometimes, but not always, reproduced by readministration of small doses of the drug to a patient who has recovered from a previous reaction, and, in certain instances, hepatic reaction to the drug

coincides with other, more obvious manifestations of drug allergy. Other phenothiazine drugs, e.g., prochlorperazine (Compazine) (109), have been implicated in reactions of this type, and several authors have pointed out that drugs unrelated to the phenothiazines can also produce this type of obstructive jaundice. The onset of jaundice due to chlorpromazine has usually occurred after the patient has been receiving the drug for one to five weeks. But the onset of intrahepatic obstructive jaundice has been reported in one patient seven days after cessation of a 109-day course of norethandrolone (Nilevar) (107). The presumption that norethandrolone was indeed the offending drug is probably valid, for norethandrolone has also been implicated as a cause of intrahepatic obstruction in several other reports. Reichel *et al.* (94) have reviewed the evidence for and against allergy as the mechanism of drug-induced intrahepatic obstruction. In reviewing the evidence, including the facts that the primary site of damage seems to be the bile canaliculi (see below) and many individuals who do not suffer jaundice develop minor aberrations of hepatic function tests while receiving chlorpromazine, we are inclined to wonder if drugs such as chlorpromazine may damage the lining of the bile channels to a minor degree in many patients and then actually sensitize the damaged area in only a few. The analogy with contact dermatitis due to drugs that are themselves primary irritants of the skin comes to mind. However, such a concept of "contact cholangitis" is highly speculative at present and ignores the many profound differences between the skin and the biliary passages.

The amine oxidase inhibitor, iproniazid, has been shown occasionally to cause a form of hepatic inflammation associated with more widespread cellular injury and necrosis. A review of 22 patients with the latter reaction (36) showed that the daily dose of iproniazid was 25 to 200 mg., the duration of treatment before the development of jaundice was two to 12 weeks, the total dose of drug given was 0.65 to 9.45 gm., and seven deaths occurred. It may be significant that three patients developed jaundice only during a second course of therapy and that the duration of the second course of therapy was only four to 14 days before jaundice appeared. Another review (99) of 90 patients with jaundice after iproniazid administration showed that the drug had been taken for a period ranging from four days to about six months before onset of the jaundice; 20 of the patients died. The hepato-cellular necrosis with inflammatory reaction caused by drugs such as iproniazid and β-phenylisopropylhydrazine (Catron) (6) is considerably more dangerous than the reaction of intrahepatic obstruction caused by drugs such as chlorpromazine. Popper & Shaffner (92) have reviewed various aspects of the classes of hepatic injury from drugs, especially the pathology of such reactions. They postulated that intrahepatic obstruction from drugs such as chlorpromazine begins with alteration in the membranes of the liver cells forming the bile canaliculi. Popper & Shaff-

ner gave useful lists of drugs implicated as causes of each class of reaction. To this list of drugs causing obstruction one may now add 4.4′ diamidino-diphenylamine hydrochloride (M & B 938) (26). Although the hepatic reaction to metahexamide was left in the unclassified group by Popper & Shaffner, this drug has been reported as a cause of hepato-cellular damage (75), and, in this instance, readministration of the drug after recovery led to accelerated reappearance of abnormal liver function tests. Although the results of such readministration are of considerable interest, one must carefully weigh the potential danger of readministration. The authors of the latter report suggest that the aminophenyl group may be important in the ability of hypolyglycemic agents, such as metahexamide and carbutamide, to cause jaundice. However, jaundice has also been reported after administration of chlorpropamide (92, 101) which does not contain the aminophenyl group; in the latter instance hepatic biopsy and laboratory tests suggested obstructive jaundice, and there were associated skin and mucosal lesions. Morgenstern (82) noted elevation of the serum glutamic-pyruvic transaminase in one of 12 patients receiving chlorpropamide. The patient also developed a papular skin eruption.

Although several phenothiazines have, as noted above, been implicated as causes of jaundice, Hollister, Caffey & Klett (58) found no cases of jaundice in 500 schizophrenic patients given phenothiazine drugs, nor in 99 given phenobarbital over a 12-week period. Five phenothiazines were used; only 100 of the patients received chlorpromazine, however. Among the 599, eosinophilia, abnormal findings in tests of hepatic function, and apparently allergic dermatitis occurred in 98, 96, and 21, respectively. The incidence of these abnormalities was not significantly higher in patients receiving phenothiazines than in patients receiving phenobarbital. The "phenobarbital control" makes this a particularly useful study.

The jaundice observed in some patients receiving novobiocin poses a special problem. It has been suspected that a yellow degradation product of the drug, rather than serious injury to the liver, is responsible for the jaundice. A recent report (23) suggests interference with the conjugation of bilirubin in the liver. This explanation was invoked to explain the genuine increase in serum bilirubin giving the indirect VandenBerg reaction, whereas other tests suggested mild disturbance of hepatocellular function.

Miscellaneous Studies and Reports

In his review of 216 patients with "drug allergy," Ljaljevic (73) included a few instances of headache, vomiting, and diarrhea. The relation of phenindione to steatorrhea has been noted in one report (63). As primary reactions, the relation of headache and most gastrointestinal reactions to drug allergy is not clear, although headache and acute gastrointestinal symptoms are not surprising concomitants of fever or severe systemic reactions, respectively. Gastric angioedema has been noted above. Ljaljevic recorded

35 instances of joint manifestations; these probably should be considered part of the serum sickness syndrome. The predominance of penicillin as a cause of the latter (31 of the 35 cases) agrees with the experience of others. Topical exposure (ointments, inhalations, etc.) was blamed in 23 of Ljaljevic's 120 patients with reactions to penicillin. The undesirability of topical use of penicillin has been stressed by many but deserves incidental mention again here. Ljaljevic stated that contact sensitization to penicillin took a relatively long time to develop in patients exposed by working with the drug, as compared to those sensitized by other methods. Andersen (3), surveying 6832 patients given penicillin, reported the over-all incidence of reactions as only 1.3 per cent. Analysis of Andersen's data suggests a significantly ($P < 0.05$) greater incidence of reactions in patients receiving benzathine penicillin (six reactions in 159 patients) than in the combined group of those receiving procaine or crystalline penicillin (73 reactions in 5050 patients). Andersen stated that an allergic background could be demonstrated in 67 per cent of the 74 patients who developed delayed reactions. The view that patients with a history of other allergies are especially prone to develop drug allergy has been accepted by many but has not always been supported by the evidence (78). Some of the disagreement may stem from the difficulty in assessing "allergic background."

The likelihood of significant exposure to penicillin in patients receiving injections from syringes containing penicillin residuals or eating various foods has continued to receive attention (86). Welch (121) points out that antibiotics are used in the preservation of poultry and fish, and in crop sprays. Penicillin was found to be present in detectable concentrations in 3.7 per cent of 1170 milk samples (61). The concentrations ranged from 0.006 to 1.22 units per ml. Tetracyclines were found in 2 per cent of the samples tested, and one sample contained bacitracin. The possibility that such sources may cause unnoticed sensitizing exposure or provoke reactions in occasional exquisitely sensitive individuals is difficult to dismiss, especially in the case of penicillin. Whereas a very rare individual may be highly sensitive to tetracyclines, the latter are not common causes of drug allergy and those who are allergic to tetracyclines are not necessarily highly sensitive to them (25).

The various eye lesions caused by antimalarials (55, 56, 67), though of considerable interest, do not appear to fall within the scope of this paper. The report of pancreatitis from chlorothiazide (62) and the appearance of abnormal findings in the chest at x-ray examination of patients receiving diphenylhydantoin (81) are certainly interesting but difficult to evaluate at present. A possible relation to collagen disease comes to mind, but the reports cited give little support to this possibility.

Treatment of Allergic Reactions

Consideration of reactions in which drugs are, themselves, the allergens must on occasion blend with consideration of the use of drugs in the treat-

ment of allergic reactions in general. Pharmacotherapy is often useful in the treatment of reactions caused by drugs, and the drugs used in the treatment of various allergic disorders may themselves sensitize the patient. Nevertheless, these two considerations are obviously not the same. The final section of this review is in effect, therefore, a separate, brief discussion of recent developments in the use of drugs in treatment of allergy.

Until recently the liberation of histamine following the reaction between antigen and antibody was considered the one reasonably well-established basis for many allergic reactions. There now exists experimental evidence suggesting that several substances are liberated during allergic reactions. Among the latter substances are serotonin (76, 118), bradykinin (70), slow-reacting substance (12), and acetylcholine (44, 114, 117). For example, it has been shown that serotonin, slow-reacting substance, and histamine are liberated from the bronchial musculature of asthmatic patients when tissue specimens obtained at operation are placed in contact with the specific antigen (103). In another experiment Tiffeneau (114) found that asthmatic patients were significantly more reactive to aerosols of histamine and acetylcholine than normal individuals. MacHaffie *et al.* (76) found serum serotonin levels much higher in allergic individuals than in normals. Vaccarezza & Peltz (117) reported that plasma cholinesterase activity was higher in allergic individuals than in normals and that the elevation of cholinesterase seemed to be proportional to the severity of the patients' symptoms. The significance of this finding is difficult to assess.

Nevertheless, the exact role played by each of these liberated substances in different allergic manifestations and the relative importance of each substance is still unknown. This may well be the fundamental reason for the relatively few real advances in the treatment of allergy that have occurred recently.

If one accepts the fact that the aforementioned substances probably do play an important role, however, one can establish an outline of possible as well as actual approaches to therapy from the pharmacological point of view. Specific desensitization, elimination of the allergen, suppression of the antigen-antibody reaction, prevention of liberation of histamine and other important intermediates, block of receptors for these intermediates, antagonism of the effects of the reaction between intermediates and their receptors, and miscellaneous methods of suppressing the "general reactivity" of the patient may all be considered.

Before considering these approaches in more detail, we must point out that many studies of the treatment of allergy suffer from lack of precision in the criteria for cure or improvement and also suffer from lack of controls. The former defect is admittedly difficult to overcome completely, but more attention to correcting the latter would, with proper experimental design, lessen the bad effect of the former. (In defense of allergists we must add the truism that studies in other fields of therapy often suffer from the same defects.) It is obviously unjustified to criticize all studies equally

and indiscriminately, and, therefore, as space does not permit detailed individual consideration of each study reported here, it is hoped that the reader whose interest is attracted by any given report mentioned below will turn to the original paper and evaluate the results after considering these highly important details of experimental design.

Specific desensitization has been useful in many allergies. Barré (4) claimed that injection of a combination of histamine and serum globulin caused improvement in many asthmatic patients and postulated that an immunity to histamine was produced. This concept, though interesting, is difficult to assess. In allergy to drugs, attempts to desensitize the patient against the offending drug have occasionally been useful but, in general, the uncertainty of good results, the possibility of dangerous reactions, and the preferability of prevention by avoiding exposure to the offending drug all combine to make specific desensitization a method that is at present seldom useful in drug allergy.

An interesting example of destruction of the allergen is the use of penicillinase. Spectacular improvement following the use of penicillinase has been reported at times (64, 116). It has been reported that patients who do not respond to the first or second dose of penicillinase will probably not respond to subsequent doses. The antigenic nature of penicillinase (120) and the difficulty in judging its effectiveness from uncontrolled studies have limited its use.

Drugs blocking the liberation of histamine and other intermediates have not achieved an established place in clinical practice at present, but experimental work in laboratory animals has been carried out in this area (70).

Blocking of receptors by antihistaminic drugs is well known, but, as Dr. Lloyd Beck has pointed out in discussions with the authors, presently available compounds leave much to be desired as regards specificity and effectiveness in blocking response to large doses of histamine. Among recent clinical reports is that of Hansel (51) who treated 225 patients suffering from chronic allergic coryza with a new preparation combining three antihistaminics with different durations of action (doxylamine, pheniramine, and pyrilamine). The duration of effect of one tablet was stated to be about 12 hours. He reported excellent results in 80 per cent of the patients. Flothow *et al.* (39) reported excellent results in the treatment of various respiratory allergies with a combination of ephedrine and the antihistaminic drug, phenyltoloxamine.

Kimura, Young & Richards (68) described a new piperazine derivative, homochlorcyclizine, with the following properties: antiserotonin activity *in vitro* and *in vivo,* antihistaminic, anticholinergic, and anti-slow-reacting substance activities, and ability experimentally to protect animals against active and passive anaphylaxis. Fisherman *et al.* (37) tried this compound in 442 patients with various allergies and reported favorable results, especially in asthma, urticaria, and rhinitis. In theory this compound should

be highly useful in the treatment of allergy, but further clinical proof is, of course, needed.

It has been claimed that gamma globulin binds histamine (4). Gamma globulin may be beneficial in patients with certain allergic reactions; Crepea & Friedlander (24) reported improvement in 50 per cent of 84 children treated with three injections of gamma globulin over two weeks. The mechanism of improvement after gamma globulin in Crepea's series could, of course, be attributable to factors not at all related to histamine binding.

Antagonism of the effects of the reaction between mediator and receptor is the principal method of therapy available to us at present, using such drugs as epinephrine, aminophylline, etc. Recent advances in this area have not been striking.

Among miscellaneous measures lessening the reactivity of the allergic patient, the use of corticosteroids and ACTH has certainly been an important advance. The more recent attempts to develop corticosteroids with a more favorable ratio of therapeutic effectiveness to toxicity have not been very successful (29, 40, 90) except for decrease in toxicity associated with mineralocorticoid effect. Prolonged use of any currently available corticosteroid in asthma and other chronic allergies has serious disadvantages (110). Therefore, one must carefully consider the indications and justification for use of corticosteroids in allergic reactions, a subject discussed in detail by Rose *et al.* (98) in an interesting paper. Where superficial skin allergies make topical therapy feasible, systemic effects of corticosteroids may be avoided (79). The ineffectiveness of corticosteroids in the immediate treatment of anaphylaxis should be noted (25) since the use of these agents in such treatment is often reported.

As psychic factors are considered by many to be important in allergy, it is not surprising that tranquilizing drugs have been used in the treatment of allergy in many recent studies (30, 48, 115). In view of the seriousness of such conditions as asthma, the frequent failure of other modes of treatment, the known ability of tranquilizers to affect certain functions of the nervous system, and the real possibility that psychic factors may at least exacerbate allergic conditions make it unjustifiable to dismiss such work in a cavalier fashion. Moreover, the effects of many tranquilizers on the autonomic nervous system may confer on these drugs a further usefulness here that is unrelated to psychic effects. But even though one must sincerely wish success to any attempt to improve the treatment of severe allergies, one may wonder whether the defects noted in some of the previous work in allergy—lack of precise criteria and lack of controls—are likely to be any less prominent as psychiatrically oriented studies become more frequent.

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METHODS FOR STUDYING THE BEHAVIORAL EFFECTS OF DRUGS[1,2,3]

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Man has exploited the behavioral effects of drugs for uncounted generations. Rauwolfia and opium have a classic history in the relief of distress, cannabis contributed unpleasantly but dramatically to politics in the Middle East in the eleventh century, and peyote and kindred materials have played their roles in religion, to mention but a few instances. Alcohol, of course, has served as a social lubricant since the beginnings of human history, to make life less dull if not less brutish and short. And the distinguished botanist, Edgar Anderson, has commented that all known sources of caffeine were discovered by primitive man and that even the prehominids probably made special use of fatigue-relieving plants (1).

Such prescientific screening for psychological activity tended to emphasize compounds that calmed undue excitement, stimulated individuals into intensified participation in events, or produced transcendental experiences; accidental discovery was the rule. Modern trends remain much the same: we still emphasize tranquilizers, energizers, and hallucinogens; happy accident continues as a major source of new psychoactive compounds.

The emphasis in this review will be on animal behavioral methods that have been used widely enough to have accumulated a reasonable body of background data to facilitate interpretation. The effects of drugs on human behavior have been covered in a recent review (2).

General Methods

Experiments going beyond the standard studies of activity and sedation emphasize conditioning, learning, and motivation. Scientific comparison of the effects of drugs on innate action patterns of a variety of species (using ethological techniques) has lagged somewhat but is increasing. Influenced by psychiatric interest in tranquilization, animal studies have emphasized effects of drugs on behavior that is under the control of aversive stimulation. This is stimulation the onset of which is punishing and the termination rewarding (e.g., electric shock), as contrasted with appetitive stimula-

[1] The survey of the literature pertaining to this review was concluded in June, 1960.

[2] Prepared during the author's tenure as a Fellow, Center for Advanced Study in the Behavioral Sciences, Stanford, California.

[3] Abbreviations used in this chapter include: CS (conditioned stimulus); UCS (unconditioned stimulus); CER (conditioned emotional response); DRL (differential reinforcement of low rates); LSD-25 (*N,N*-diethyl-D-lysergamide).

tion in which the onset is rewarding and the termination possibly negative in effect (e.g., food or water reinforcement) (3, 4, 5). Most workers recognize that a substance will be unlikely to influence emotional behavior significantly without influencing other kinds as well and that the behavior of an organism—including pathological behavior—is a totality that does not readily permit easy fractionation to produce specific effects without some risks of generalized consequences. Accordingly, studies of largely nonemotional behavior, as well as of emotional behavior, have been quite common, not only to get information on behavioral effects of drugs in general but also to permit an assessment of "behavioral toxicity"—the general psychological cost in adaptation and performance that a substance may exact for producing some desired, specific effect (6, 7). Further, important new findings about behavior itself are beginning to emerge from attempts to develop test methods sensitive to behavioral effects that familiar drugs are thought to have.

Experimental Neuroses

The production of experimental neuroses in animals is an appealing technique for investigating effects of drugs and other therapies on complex, emotionally toned behavior. These persistent, maladaptive behavior patterns can be produced almost at will under controlled laboratory conditions, and the symptomatology resembles that found among human beings with emotional-motivational disorders (8). Russell's survey noted increased irritability, increases or decreases in spontaneous motor activity, phobic behaviors ("irrational fears"), regression and loss of learned adjustive habits, posturing and tic-like movements, abortive occurrence of conditioned responses at inappropriate times (hallucinations?), changes in social behavior toward increased aggressiveness or toward a withdrawn and submissive pattern, plus effects presumably of autonomic origin such as altered respiration, rapid and irregular pulse, and hypersensitivity to stimuli as characteristic symptoms (9). In Pavlov's original experiments (10) and in later studies by Liddell & Bayne (11) and others (see 12, 13), such behavior was thought to develop when the animal was forced to make discriminations beyond its capacities. Subsequent work has revealed that other demands and constraints on the animal can produce breakdown: restraint of voluntary movement, unavailability of alternative possibilities for response, insoluble problems with punishment for failure, demands for protracted inhibition, forced alteration in patterns already learned, strong painful stimulation, and conflict situations in which the animal is forced to experience an unpleasant stimulus in order to get an earned reward (9).

Liddell and his colleagues (14, 15), and Anderson & Parmenter (16), working largely with sheep, goats, and dogs, have added self-imposed restraint and monotony to this list. Initially, they employed the Pavlovian harness to restrain the animal and conditioned leg flexion to the sound of a metronome with reinforcement by unavoidable shock to the leg as the unconditioned stimulus; more recently, they have allowed the animal free

movement in the experimental room and have supplemented each leg shock with 10 seconds of prior darkness. The rigid temporal schedule of stimulation and conditioning trials they considered a critical condition for development of neurotic behavior, with the daily pattern of anxiety-producing monotony consisting of a series of 20 conditioned signals for darkness (and shocks) spaced exactly two minutes apart. Liddell has argued that the sheep or goat in training eventually substitutes a self-imposed restraint for the external restraint ordinarily imposed by the laboratory harness. The animal thereby becomes "emotionally muscle-bound" and in a "psychical straightjacket of anxious foreboding" so that it can no longer cope with the vicissitudes of daily living, in or out of the laboratory (15, p. 103), in a manner reminiscent of the human patient incapacitated by excessively rigid self-imposed restraints on thought and behavior.

Both Russell and Masserman emphasize the importance of conflict. Russell (9) argues that conflict in some form is to be found in all neurotogenic situations and that all limit the animal's possibilities for escape from a conflictful situation that generates competition between incompatible response tendencies. Masserman and his colleagues have elaborated conflict techniques to produce neuroses in the cat and monkey, as part of a program of basic research in psychiatry (17, 18). In addition to observation and rating of spontaneous individual and social behavior, Masserman's methods include two- to eight-months training until the animal has learned to respond efficiently to specific visual, auditory, olfactory, or temporal stimuli by pressing one of two available levers a required number of times to secure a reward such as food, warmth, liquid, sexual gratification, etc. The data from observation and training, supplemented by continued observation of the subject in and out of the apparatus, provide baselines for evaluating learning, what the subject's normal behavior is like, and a picture of its adaptive patterns and their variation under "normal" laboratory stress. Neurotic breakdown is then induced by presenting the animal with an aversive stimulus (e.g., a shock or air blast for the cat, a toy snake or loss of a companion for the monkey) just as it is getting its reward for the learned behavior. From three to 40 or 50 repetitions of this experience produce dramatic departures from normal behavior and a loss of learned adaptations (see 19).

Recently, Masserman has summarized over 20 years of this work to show that experimental neuroses respond favorably to treatment with psychoactive drugs, as well as to other treatment (20, 21). Though alcohol adversely affects learned adaptive skills, particularly the most complex and recently acquired, it also disorganizes the complex neurotic behavior to allow simpler, learned adaptive patterns that have been disrupted by the neurotic symptoms to re-emerge, with beneficial net effects on over-all behavior. Morphine and barbiturates also relieve experimental neurosis, somewhat more effectively and with fewer side-effects than reserpine, chlorpromazine, meprobamate, and other tranquilizing drugs.

However sensitive they may be, grossly, to drug effects and however useful as rough screens for psychological activity or as demonstrations, experimental neuroses are usually difficult to use with real precision as a tool of analysis. The behavior iself is complex. The conditions necessary and sufficient to produce it are in dispute, and, as listed, cover quite a range of experimental possibilities. That an experimental neurosis is alleviated by a drug tells little, by itself, about the specific actions of the substance—it might be altering any one of a complex chain of interdependent events to produce the dramatic effect. Further, theories about human personality and psychopathology, given their present confused state, in the face of difficulties imposed by interspecies differences, help little if at all in the scientific understanding of experimental neuroses.

Wolpe (13) has argued persuasively that the situation can be simplified, contending that production of these neuroses depends only on the presence of one of two factors—(*a*) noxious stimulation or (*b*) "ambivalent" stimulation—acting in the presence of (*c*) confinement. Noxious and confinement have their usual meanings, but here "ambivalent" is more complex and refers to a stimulus situation that tends to evoke opposing responses simultaneously in approximately equal strength. Though this appears to resemble "conflict," the meaning is closer to "ambiguous." For example, if a dog is conditioned positively to salivate to the conditioned stimulus of a circle and negatively to inhibit salivation to an ellipse with semi-axes in the ratio of, say, 2:1, presentation of an ellipse of intermediate oblateness, with semi-axes in the ratio of 9:8, will evoke, simultaneously, both a tendency to salivate (by generalization from the circle) and a tendency to inhibit salivation (by generalization from the 2:1 ellipse). If the opposed tendencies are approximately equal in strength and if the animal must cope with this stimulation repeatedly under conditions of confinement, Wolpe believes neurotic breakdown should ensue. This view resembles Pavlov's original emphasis, except that Wolpe rejects Pavlov's idea that the clash between excitation and inhibition necessarily produces a physical change in cortical neurones. Rather, along with most other workers, he considers that neuroses are learned and can be altered by new learning (see also 22).

"Ambivalent" stimulation would thus explain those experiments in which breakdown developed out of difficult discriminations, threshold stimulation, prolonged inhibition or delay of reinforcement, and rapid alternation of positive and negative stimuli. In most "conflict" experiments, however, the animal's incompatible and opposed response tendencies arise from the requirement that it take an unpleasant stimulus (which established escape and avoidance) in order to gain a valued reward (which has reinforced approach). These, as well as the Liddell experiments in which the conditioned reflexes were reinforced by shocks, Wolpe would consider as instances of noxious stimulation. Here, the conditioned stimuli introduced by the experimenter, plus stimulus aspects of the experimental situation, have been paired directly or indirectly with painful stimulation to acquire,

through conditioning, the power to evoke emotional disturbances as conditioned responses. This view coincides with that expressed by a number of investigators concerned with conditioned fear and anxiety (e.g., 23, 24, 25)

Wolpe handles monotony as a neurotogenic factor in the Liddell experiments by pointing out that monotony alone, unaccompanied by traumatic stimulation, rarely if ever produces breakdown among animals in other contexts. Neurosis probably developed more easily under rigidly scheduled stimulation (even when all positive) simply because the waiting-time interval between trials became a conditioned stimulus for emotional response; the interval, too, always preceded and, thus, always was paired with the shocks so that the animal was continuously subjected to conditioned aversive stimulation. [That time intervals can become effective conditioned stimuli is, of course, well known (26.] This observation contains an important caveat for all behavioral experimentation: the subject, animal or human, does not necessarily learn just what the experimenter intends, nor is conditioning necessarily confined to only those stimuli deliberately presented!

The necessary conditions for producing animal neurosis probably can and should be reduced still further. First, Wolpe's notion of "ambivalence" as a primary neurotogenic factor may have a technical weakness. Generalized conditioned responses, both positive and inhibitory, grow sharply weaker as the evoking test stimuli diverge from the values employed in the original training. If the original training and differentiation has been well established, the strength of the opposed positive and inhibitory response tendencies could be expected to be at rather low levels in the case of intermediate (and thus "ambivalent") stimuli—perhaps at too low a level for the clash between them, as such and by itself, to make a really substantial contribution to total behavior. Second, the behaviorial potency of these situations may stem not from their "ambivalent" character but from their aversive properties. Indeed, Hearst & Sidman (27) have presented data showing that escape from concurrent positive and negative stimulation is rewarding, indicating that such situations have noxious (aversive) properties. Thus, only two factors may be critical: noxious stimulation (to reinforce the conditioning of emotional responses to whatever stimuli are present in the situation) and confinement (to keep the subject in the situation so that the unpleasant stimulation can have its full conditioning and other behavioral effects).

Conditioning and Learning Techniques

Conditioning and learning techniques that allow isolation and control of critical components of behavior probably offer greater promise as analytic tools than more diffuse methods, at our present stage of development. The words isolation and components here do not imply testing a restrained animal that can move only a single limb or sense through only one modality. Far from it; the total behavior of the unrestrained organism is probably most sensitive to experimental effects. Rather, these goals are accomplished

through procedures that permit the investigator to determine what actually happened and of what it was a function, both within a single experiment and across experiments made comparable by appropriate controls (28).

The simplest of these techniques is not far removed from naturalistic observation. A neutral signal (the CS) is simply paired repeatedly with an aversive unconditioned stimulus (UCS), usually an electric shock, until the CS acquires the power to evoke a conditioned response, which usually is of emotional character with escape components. Watson & Raynor (23) followed this procedure in conditioning a child to fear furry objects by associating them with a loud sound; later investigators have employed the method in studies on the effects of drugs and other variables (29 to 32). In fact, Wolpe found this the more effective of his two neurotogenic methods and produced, in cats, strong conditioned emotional disturbances that became evocable by a wide variety of stimuli, including the experimenter and the laboratory situation (13). Such conditioned emotional responses (CERs), if strong, appear as a pattern of behavior, easily identified by the experienced observer, that is characteristic of the species, e.g., inhibition of gill movements in goldfish (33), urination and defecation, immobility, cowering in remote parts of the experimental chamber, running or jumping (34), vocalization, attempts to escape or attack. This kind of CER conditioning is quick and cheap, and the learning is durable in the sense that the conditioned response can be evoked by the CS long afterward without any intervening training or practice. Several studies have found that chlorpromazine interferes with acquisition of the simplest form of the CER in the mouse and rat, with amount of interference somewhat dependent upon dosage, though the data do not agree on whether the drug facilitates, retards, or has no significant effect upon experimental extinction (25, 34, 35, 36).

Without substantial modification in method, such simple conditioning has insufficient sensitivity and reliability for precise work. Conditioned emotional responses (as, indeed, all responses) must be considered to appear as and to be defined by a change in the quantity or kind of behavior being emitted intercurrently by the subject, or both. For CERs so weak that the characteristic gross patterns of emotional reaction do not emerge, the conditioned response may appear only as a slight slowing in eating or reduction in exploratory movement (or whatever else the animal is doing in the apparatus), and its occurrence may be missed. Similarly, small differences in the intensity of the response are hard to discriminate, particularly if the responses are weak. Quantitation of the ongoing behavior interrupted by the CER can offset these difficulties to some extent by objectifying and bringing out small deviations indicative of the conditioned response (37). Unless this ongoing behavior is under precise experimental control, however, incidental changes in it produced by general effects of a drug or other factors can obscure the conditioned response or alter its threshold and thus alter the sensitivity of the test.

Superimposition of this aversive conditioning upon some other control-

lable learned behavior, as reported in a number of investigations (25, 29, 30, 31, 37 to 42) objectifies the reaction, provides a means of adjusting sensitivity, and permits a monotonic (though probably not linear) measure of its intensity. Typically, in these studies instrumental lever pressing for a food or water reward was first established; then, CER conditioning was superimposed upon the regular output of lever responses by three- to five-minute presentations of the CS terminated by shocks to the feet during lever-pressing runs. After a few such pairings, presentation of the CS reduces or stops the output of lever responses, even though no lever response has ever been punished directly by a shock. Decrease in output during the CS relative to output of lever responses during an equivalent prior interval indexes the occurrence and intensity of the CER or "conditioned suppression" (40, 43).

In the earliest application of this technique to studies of drug effects, Wickler *et al.* (44) and Hill *et al.* (45, 46) showed that morphine weakened the CER in the rat, indicating the beneficial effect of this substance on the relief of anxiety associated with the anticipation of pain (see also 47, 48). Subsequent studies on the same species have showed that meprobamate (37) and reserpine (41) tended to weaken a CER established under non-drug conditions, with reserpine having by far the greater effect. In fact, reserpine also abolished the usual rise in plasma steroids normally found with evocation of the CER, as the CER was weakened by the drug (49, 50). Chlorpromazine also weakened a CER superimposed upon an instrumental habit in which the rat ran from one end of an experimental chamber to the other to get a water reward (51). In this study, Heistad also was able to show that chlorpromazine had the opposite effect—increased the strength of the CER—if that conditioned response had first been weakened by electroconvulsive shock. Though this latter effect of chlorpromazine was small, it was clear-cut and contrasted in an interesting fashion with other experimental data indicating that meprobamate further weakened a CER previously attenuated by electroconvulsive shock (52).

Though CER conditioning superimposed on lever pressing produces rather similar effects in organisms as diverse as the rat, cat, and monkey (42, 53), species differences requiring further analysis have emerged. For example, Valenstein (54) has reported that the guinea pig shows only a moderate decrease in lever pressing but omits to collect the water rewards during presentation of the CS in CER conditioning with shock intensities that render the rat completely immobile. More important, reserpine appears to have quite different effects on the CER in the two species. In the rat, this drug normally weakens the CER, decreasing the suppressant effect of the CS to increase lever pressing to almost normal rates for that run during presentation of the CS. In the guinea pig (which is more sensitive to that drug than the rat) the effect is the reverse: the suppressant effect of the CS (and the intensity of the CER, as indexed) is increased as compared with placebo runs.

Initially, the CER seemed almost ideally suited for the study of drug effects on emotional behavior. The basic conditioning appeared to be of the simplest kind—just pairing of signal and shocks—with the strength of the CR easily manipulable through variations in number and distribution of the pairing and in the intensity of the shocks. Similarly, the variations in the amount and frequency of food or water reward and in the level of prior deprivation influence the strength of the "competing" lever response to permit control over the sensitivity of the test. Finally, the indicator was not only objective, but also could be used to demonstrate the selectivity of drug effects when the CER was altered while the lever pressing remained essentially intact.

Though the technique has proved useful, the peculiar nature of the "competition" between the lever response and its suppression by the CER has complicated both measurement and interpretation, with a clear resolution of all difficulties not yet in view. For example, the speed with which suppression is acquired in CER conditioning is uncorrelated with rate of lever pressing, as such (55). However, the relative frequency (proportion) of lever responses rewarded distinctly influences both speed of acquisition (56) and speed of extinction (57), with the CER harder to establish and easier to extinguish the larger the proportion of lever responses rewarded. The competition appears to be between reward and emotional disturbance (between two motivational systems?). Stein *et al.* (58) have presented data to indicate that the extent of the suppression is influenced decisively by the degree to which it reduces the rat's opportunities to get the reward for pressing (rewards that could be earned, and received, if the rat pressed the lever, but which are "missed" when the animal does not press during the CS). Other workers have suggested additional complications related to the rat's abilities to discriminate changes in the relative frequency of reward (59). Geller (60) has demonstrated that the speed of acquisition and extinction of the CER depends in part upon the character of the reward for lever pressing, with the suppression developing more slowly and extinguishing more rapidly if sweetened milk rather than water rewards the lever response. Also, suppression does not appear if the lever response is maintained by brain stimulation as the reinforcer (61). Further, Halasz & Hunt (62) have reported that when the CER was overtrained (by giving the rat 100 conditioning trials, as compared with the usual two or three to 20 or so), the CS lost its control over the suppression and no longer produced it. Apparently, the suppression was disseminated to appear fractionally and sporadically throughout the entire run, whether the CS was on or off. Paradoxically, these overtrained CERs, that had been reinforced many times by shocks, extinguished extremely rapidly, as compared with CERs based on five or six pairings of CS and shock. That the CS had retained psychological significance through all this overtraining—had not "adapted out"—was indicated by the fact that nonoccurrence of the CS at the regular time in a lever-pressing run tended to restore discriminated suppression on the

next trial. Finally, Sidman's recent, elegant review (63) summarizes studies in which interactions between the CER and other aversively controlled behavior decisively influenced the dynamics of both, even whether any suppression at all occurred. Among other things, these studies show that the simultaneous use of two different behaviors may complicate the analytic situation as much as the simultaneous use of two different drugs (see also 64).

Instrumental Avoidance Conditioning

Instrumental avoidance conditioning has been one of the most widely used aversively controlled behaviors for drug studies. Here, in the presence of a signal indicating the imminence of a shock or other noxious stimulus, the animal learns to make some specified response, first to escape the shock and, later, to forestall it. The response required may be climbing a rope, jumping up on a "safe" platform or piece of hardware cloth inclined at a steep angle to the floor, leaving a to-be-charged chamber of an apparatus for another section, turning a wheel, pressing a lever, or whatever is appropriate to the species and to the expected motor and psychological effects of the drug (see 65 to 68). The noxious properties of the shock motivate escape, and termination of the pain reinforces this response, but the avoidance response that grows out of the escape behavior and which eventually comes to anticipate the shock and prevent its occurrence is probably not conditioned as a reflex to the CS by virtue of the simultaneous occurrence of escape and the conditioned stimulus. In fact, an animal initially given pairings of CS and shock in a situation permitting no escape can later acquire an avoidance response if reinforced only by termination of the CS (and other stimuli associated with the situation in which shock was given), without further shocks (24, 69). The CS, through pairing with shock in what is essentially CER conditioning, acquires psychological properties that disturb the animal and that make termination of the CS rewarding. At the higher levels of apologetics in behavior theory, views differ as to the full explanation of avoidance conditioning. Some workers (e.g., 24, 70, 71, 72) contend that the response of "fear" or "anxiety" that the CS has acquired the power to evoke also has drive or motivational properties—that the avoidance response is reinforced by a reduction in this "learned drive" as the animal gets out of the stimulus situation that evokes it (i.e., makes the avoidance response). Others (e.g., 4, 73, 74) consider that the CS has acquired aversive properties through conditioning so that its termination reinforces avoidance. Other intermediate views and alternative positions have been proposed (75, 76). For many experiments, these varying opinions make no difference and can be disregarded. For others, however, they can pose critical empirical and inferential problems, particularly if the experimenter wishes to argue that the drug effect on avoidance is mediated through a change in motivation or drive. To make such an argument firm requires additional observations (77).

Miller *et al.*, in an excellent study (78), contrasted avoidance and escape to show the weakening effects of chlorpromazine on avoidance. All drug tests for effects on avoidance came during extinction, after conditioning had been completed. Two experimental situations provided for the contrast: a double-grill box, for avoidance, and a long alley with a continuously electrified floor that the rat had to traverse to escape the shock, to control for motor effects of the drug and for responsiveness to pain during the drug tests. The data show that chlorpromazine clearly reduced the number of avoidance responses obtained during extinction, with a clear dose-effect relation. These investigators controlled for motility by first determining the effect of 1.25 mg./kg. chlorpromazine on running time in the alley and then determining that dose of phenobarbital which would produce equivalent slowing in the running of other trained rats. Then, trained rats under each of these drugs, in doses of equal potency with respect to effect on alley performance, were tested in the avoidance situation where chlorpromazine clearly weakened avoidance while the "equivalent" dose of phenobarbital had little effect. With similar care (and a similar design), these authors (79) also investigated the persistence of the effects of chlorpromazine on experimental extinction (effects of a single dose had been found to be reversible, while several consecutive doses appeared to have more durable effects). With controls for cumulative effects, as such, of the drug and for varied intervals between training and testing, they found that chlorpromazine potentiated experimental extinction and that such extinction under the drug was as permanent as extinction under placebo.

Other investigators have compared escape and avoidance, with both responses having the same topography (i.e., physical characteristics) to show stimulating effects of drugs or to show that a drug such as chlorpromazine weakened or eliminated the avoidance reaction, though the animal still could feel the shock stimulus and still retained sufficient motor function to make the physical response (80 to 83). Verhave *et al.* (84), using an avoidance and an escape response in which the rat rotates a wheel to terminate the CS and to avert or terminate the shocks, proposed a comparison of percentage loss of avoidance with percentage loss of escape behavior to distinguish, in a continuous test, between general depressant effects of tranquilizing and sedative drugs on both responses and specific effects on avoidance. More recently, Weiss & Laties (85, 86) have described a most elegant and precise method for titrating avoidance and escape behavior, based on the observation that rats will press a lever to produce small decrements in the intensity of electric shock to the feet. In testing escape behavior, if the lever is not pressed, the shock periodically increases in intensity, whereas regular and diligent performance can keep it at a minimum. In testing avoidance behavior, the shock is administered intermittently in bursts of increasing intensity if lever responses are not made; lever responses in the intervals between bursts, however, are rewarded by a reduction in the next shock from its last previous value. Comparable behavior, in different subjects, with the important gen-

erating parameters subject to precise control, can be produced as a function of a shock that is present or as a function of a shock to come. Further, this test, like Verhave's, is continuous rather than episodic, as are so many avoidance tests, and permits the time course of a drug effect to be plotted quite exactly.

The use of avoidance conditioning in combination with behaviors controlled by appetitive reward allows analysis of differential effects of drugs on different kinds of habits. For example, John *et al.* (87, 88), in a study of the effects of intraventricular and intramuscular injections of reserpine and other drugs in the cat, employed three different behavioral indicators: running on an elevated pathway and crossing a hurdle to get food, a two-choice discrimination problem with food reward, and avoidance in a double-grill box. They found reserpine via both pathways weakened avoidance but left runway approach behavior relatively untouched; in small doses, this drug interfered with avoidance to a visual but not to an auditory signal. Hughes & Halasz (89), using a technique in which cats were trained intercurrently in the same apparatus to press a lever for milk reward and to deflect a pendulum (on signal) to avoid a shock, found that amphetamine disrupted the appetitive lever response but left avoidance untouched. Halasz (90), in the same situation, found that chlorpromazine weakened avoidance but did not influence lever pressing for milk. Ray & Stein (91) have presented preliminary data for the rat (using a situation in which the animal pressed one lever, on signal, for milk and another lever, on a different signal, to avoid shock) to indicate that reserpine, chlorpromazine, LSD-25, and meprobamate selectively weakened avoidance, while amphetamine selectively eliminated lever pressing for milk. Wenzel (92), in a similar situation, found reserpine weakened avoidance in cats.

Miller and his co-workers (e.g. 93 to 96) have used compound test situations ingeniously for many years in the study of conflict and the effects of various procedures upon it. Miller pits an approach habit (e.g., running down an alley to get food) against avoidance tendencies set up by aversive stimulation (e.g., shock) given at or on the way to the goal. Conflict appears as vacillation between approach and avoidance tendencies, increased running time, and the like. Strength of approach and avoidance tendencies (and thus strength of motivation) were inferred from running speed, strength of pull on a harness, or some other suitable score. With proper controls and computations, selective effects of drugs on the components of the conflict, including motivation, can be analyzed. Miller and his group have been very productive in developing variants of this basic situation and adapting it to experimentation on drugs. His recent, forceful reviews cover this program (97, 98, 99) and new aspects of his theoretical views (100) in detail.

One of the most serious problems to be solved in all experimentation using compound tests is to make sure that the different behaviors to be compared are approximately equal in strength prior to introduction of drugs, or to provide some correction for inequality. Otherwise differential effects

of a drug on the several habits may reflect only differences in their relative strengths, rather than real selectivity of effect, as desired. In many experiments employing different sustaining motivations for behaviors in different experimental settings which require different physical responses, such equality is hard to estimate, let alone establish firmly. The solution of conditioning several habits intercurrently in the same apparatus and requiring similar or identical responses for each may be helpful but is not entirely satisfactory. Such habits appear to interact, and probably in proportion as the physical responses for each approach topographical identity. Finally, what aspect of strength is to be equalized? The word and concept appear ubiquitously in discussions about behavior, yet the standard indicators of strength such as latency, rate, amplitude (intensity), and resistance to extinction tap different aspects of a habit and, thus, do not always agree.

In an exciting series of experiments, John & Killam (101) have explored electrophysiological correlates of the process of simple avoidance conditioning. Recording electrodes were implanted in a number of loci in the brain of the cat, including cortex, midbrain tegmentum, lateral geniculate body, superior colliculus, fornix, septum, amygdala, hippocampus, and nucleus ventralis anterior of the thalamus, with placement confirmed histologically at the end of the experiments. After postoperative recovery, the cats next received adaptation ("familiarization") trials in the double-grill box, with bursts of 10/sec. flickering light introduced to allow a determination of baseline electrophysiological response on all leads to that stimulus. Then, the cats were subjected to avoidance conditioning, with the 10/sec. flickering light serving as the CS and intermittent shocks as the UCS and with the apparatus illuminated by steady light of equal intensity between trials. The flickering CS provided a tracer frequency that could be recovered electrophysiologically from the various brain structures during adaptation and subsequent avoidance conditioning. Such "labelled" electrical responses appeared during adaptation in the visual and auditory cortex, lateral geniculate, superior colliculus, amygdala, and hippocampus, but soon disappeared. They reappeared, except in the hippocampus and amygdala, with the first pairing of CS and shock in conditioning. As training progressed, the labelled frequencies first left the classical visual system to appear in the extralemniscal ascending system and then went back to the visual pathways and centers again toward the end of training. When the cats had achieved perfect performance, 40/sec. bursts appeared in the amygdala and multiples of the 10/sec. CS frequency were found in the cortex. As reserpine blocked the avoidance behavior, the electrophysiological picture regressed to that found in early and intermediate stages in training; as the cats recovered from reserpine, the electrical picture at any given performance level resembled that seen at the equivalent performance level during training. A variety of generalization experiments in most instances confirmed the specificity of the electrophysiological findings to the overt behavior data. These experiments,

with extensions now in progress, signal real progress indeed in an important field of inquiry.

Operant Conditioning and Nondiscriminated Avoidance Conditioning

The operant conditioning methods of Skinner (3, 4, 5) and nondiscriminated avoidance conditioning, developed within the operant framework by Sidman (and justly called "Sidman avoidance") (63, 102, 103, 104), have contributed most fruitfully to the investigation of both behavior and drug effects. Recent reviews and summaries (43, 105 to 110) cover major contributions and considerations of philosophy and technique in detail, making extensive discussion here unnecessary.

Operant behavior is controlled by its consequences; the governing reinforcement is correlated with the response (hence, Type-R conditioning). Any response so controllable, that can be made to occur at a convenient rate, and that, when completed, leaves the subject in a state and position where it is free to respond again and again can be used as an operant (e.g., lever pressing for food reward) (111). The other important category is respondent behavior, established by Type-S conditioning in which the reinforcement (UCS) is correlated with a stimulus (CS) (e.g., the CER, established simply by pairing a CS with shocks).

Operant investigations on drug effects uniquely exploit special schedules of reinforcement, particularly when behaviors under appetitive control are involved. The conditioning apparatus can be set to reward each lever response (continuous reinforcement) or to yield intermittent reward in accordance with an automatically controlled schedule. The schedules may be arranged to make reinforcement available (if the subject presses the lever) (*a*) only after the lapse of a constant or variable interval of time (fixed and variable interval schedules), (*b*) only after the subject has made a specified number—constant or variable—of responses (fixed and variable ratio schedules), (*c*) only as the subject holds its output down to some predetermined low rate [differential reinforcement of low rates (DRL)], and so on. In DRL, only those responses that follow the last preceding response by some predetermined interval (say, 10 or 20 seconds) are rewarded. Operant behavior extinguishes, of course, if reinforcement is continuously withheld.

Different schedules produce characteristically different output curves, defined in terms of rate and distribution of responses in time. Different kinds of schedules can be combined, almost on a prescription basis, to create a behavioral situation with the desired psychological features and spectrum of outputs (e.g. 106, 107, 112, 113). Different kinds of output can be placed under stimulus control by giving one signal (e.g., a light) when one schedule is in force, and another (e.g., a sound), when another schedule is in force, and so on, so that each signal becomes the occasion for the occurrence of the appropriate output. Sequential presentation of several different

schedules (with signals) within a single run tests for differential effects of a drug on several different kinds of behavior, with selective effects demonstrated if one kind is spared while another is disrupted. Alternatively, different schedules may be combined sequentially in this way, but without any previous association with exteroceptive signals, to produce different kinds of output during a single test. Here, in place of special lights or sounds, the subject must depend upon stimuli from his own behavior and its consequences for the signals as to which schedule prevails, to make what Dews has found to be a most sensitive test for drug effects (e.g., 106, 114, 115, 116).

Inferences as to what it is the subject is discriminating as a signal or as to what behavior is being reinforced or blocked, and by what, plays a critical (though insufficiently emphasized) role in the analysis of drug effects on operant behavior. For example, with DRL schedules, elapsed time since the last previous response must be discriminated if the rat is to delay his next response long enough to get a reward. With DRL, Sidman (117, 118) found that amphetamine increased the rat's output but broke up the timing discrimination, while alcohol reduced output but left the timing discrimination intact. Blough (119) trained pigeons to remain still in one place by making the probability of getting a reward from the food magazine increase as the bird stood still for longer intervals. Chlorpromazine increased the ability of the subjects to sustain this continuous response, and pentobarbital weakened the response and made them more active. Presumably, chlorpromazine tended to counteract emotional disturbance generated by the active inhibition the response demands, to improve performance.

In nondiscriminated (Sidman) avoidance, the subject learns to press a lever (or make some other suitable response) to postpone a shock for a response-shock interval of, say, 10 seconds. If the lever is not pressed within a specified interval after a shock, again 10 seconds, the subject receives a shock. This shock-shock interval and the response-shock interval, are the major parameters governing the behavior with some contribution from shock intensity (120).

Opinions differ as to how, exactly, avoidance lever pressing should be explained (63, 121); the technique, however, has both stimulated discussion and revision in behavior theory and opened the door to more efficient investigation of drug effects on aversively controlled behavior. Here, the indicator behavior can be controlled with precision and adjusted in sensitivity. The technique can yield more data per unit of running time than traditional avoidance methods requiring that the subject be returned either to a starting position for the next trial or to an intertrial waiting period. Further, it can be combined with appetitively controlled behavior as one of a sequence of schedules presented during a single run to bring out selective drug effects. As an example of its sensitivity and subtlety, Sidman found that 0.1 mg./kg. reserpine sharply reduced avoidance lever pressing if the rat was given only about 20 per cent of the shocks it should have gotten for

failing to press diligently and regularly enough to forestall them, but the effect disappeared if the rat received all the shocks it "earned" (122). And Verhave (123) has used the Sidman technique to show that amphetamine increased rate of avoidance responding but that this increase was not attributable solely to increases in general activity.

Recently, Brady (124) has described what is probably the most systematic application of complex multiple schedules in assessment of drug effects on the behavior of the rat and monkey. In his "drug-behavior profiling program," he maintains panels of trained subjects of each species receiving daily four-hour test runs which consist of a fixed sequence of four different reinforcement schedules under the control of external signals. Performance of these trained subjects on each schedule under nondrug conditions provides baseline data for each kind of behavior, including Sidman avoidance; departures from these baselines under drugs can indicate selective or generalized effects, depending upon which components are affected, and how. Brady's detailed analysis of the behavioral changes produced by scopolamine and pentothal not only brings out the sensitivity of the indicators and the selective effects of the drugs but also illustrates something of the complexities encountered in interpretation of operant data.

At least some of the behavior of most animal species can be brought under operant control, though ethological peculiarities in some instances pose difficult problems [see (125) for an engaging account of attempts to condition the octopus]. Operant methods have been applied to the study of depth of sleep (126), stuttering (127), human-observing and signal-monitoring behavior (128, 129), child development and behavior (130, 131, 132), and human psychosis (133, 134), among other diverse problems (5). Many different kinds of stimuli have reinforcing properties, including vibration (135) and tactile stimulation (136), onset of illumination (137), attention (131, 138), noise (139), the sight of new or interesting objects outside the apparatus (140), and electrical stimulation of the brain (141, 142), to mention but a few.

Olds and his co-workers have made self-stimulation of the brain (both electrical and chemical) the basis for an extensive program of investigation of drug effects on behavior. Here, operant lever pressing is acquired and maintained in the rat by reinforcement with electrical stimulation through microelectrodes implanted in various hypothalamic or limbic system locations. Presumably, this stimulation activates hypothetical reward ("pleasure") systems in the brain (or, in other locations, "pain" systems with unpleasant or ambivalent effects leading to fluctuations in output of lever responses). Some of the experiments are complex and an adequate picture of the program can be gained only by careful reading of the recent articles and summaries which cover the details (143 to 147). These workers have accumulated considerable evidence, however, to indicate that the two systems respond differentially to drugs and that the method is powerful and productive. For example, some drugs, (e.g., meprobamate, pentobarbital, and

morphine) inhibit operation of the "pain" system but leave the reinforcing function of stimulation of the "pleasure" system relatively intact, while chlorpromazine has the reverse effect.

What identity these "pleasure" and "pain" systems have with well-known CNS functions defined by more traditional physiological and anatomical methods is uncertain. Similarly, the behavior maintained by intracranial self-stimulation may not turn out to be entirely identical with an operant maintained by giving the deprived animal food or water rewards. Here, as is so common in the science of behavior, the empirical findings may indeed have outrun our capacities for explanation. Such an outcome could be a stimulating challenge leading to healthy developments in psychology, pharmacology, and physiology, however disconcerting it might be momentarily.

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BEHAVIORAL PHARMACOLOGY[1,2,3]

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Introduction

The intense interest in behavioral effects of drugs in recent years has led to an unusual number of symposia and reviews. Much of the most significant work through 1955 has been reviewed by Wikler under the title of *The Relation of Psychiatry to Pharmacology* (148), and selected topics are reviewed in *Psychopharmacology: Problems in Evaluation* (24). Many reports of conferences or symposia have appeared (5, 13, 14, 28, 30, 40, 49, 70, 71, 72, 85, 104).

The "Assessment of Tranquilizers" has been reviewed by Riley & Spinks (111), "Motivational Effects of Drugs" by Miller & Barry (88), and "Behavioral Pharmacology" by Sidman (119). In the *Annual Review of Medicine* some of the older work on the reticular formation of the brain stem has been surveyed under the title of "Psychopharmacology" (48), and in the *Annual Review of Psychology* series, "Psychopharmacology" has been reviewed by Ross & Cole (113). A relatively exhaustive account of the pharmacology of drugs distinguished for their behavioral effects is given, in German, in the volume by de Boor entitled *Pharmakopsychologie und Psychopathologie* (10). Therapeutic use of the newer drugs has been reviewed by Cole *et al.* (25), and the toxicology of tranquilizers is presented in a volume edited by Grebe (54). In addition, the Psychopharmacology Service Center periodically makes available to investigators exhaustive annotated compilations of the titles in specific areas of psychopharmacology.

Today, behavioral pharmacology is in the condition of physiology early in the nineteenth century when in 1837 Grainger remarked, "There is probably no branch of knowledge which, at the present day, offers so large a collection of insulated facts and which is yet so defective in general prin-

[1] The survey of the literature pertaining to this review was concluded in July, 1960.

[3] Following is a list of some terms that may be unfamiliar to the reader, with reference to the page in which they are explained: Aversive stimulus, page 149; Avoidance behavior, page 150; Battery of tests, page 161; Continuous avoidance, page 163; Conditioned suppression, page 158; DRL schedule, page 168; Discrete avoidance, page 163; Escape behavior, page 150; Multiple schedule, page 153; Neutral stimulus, page 158; Schedule, page 152; SMA (spontaneous motor activity), page 148.

Abbreviations used in this chapter include: CPZ (chlorpromazine); UR (unconditioned response); CR (conditioned response).

[3] The preparation of the manuscript was supported, in part, by a grant from the U. S. Public Health Service (M 2094) and by the Eugene Higgins Trust.

ciple, as physiology" [quoted by Liddell (83)]. This makes the selection of area for coverage for a short review unusually arbitrary. This review will be limited to discussion of results of experiments relevant to the objective assessment of the effects of drugs on the behavior of more or less intact animals, and to only two classes of drugs: tranquilizers and amphetamines. In general, the starting point has been the review of Wikler (148).

Major Tranquilizers

Effects on unconditioned behavior.—Chlorpromazine (CPZ) and related drugs protect against the enhanced lethality of amphetamine to grouped mice. The toxicity of amphetamine to mice is greatly increased if the mice are aggregated following injection of the drug (see later under "Amphetamines and Related Compounds"). The LD_{50} of amphetamine was reduced from 111 mg./kg. when the mice were put singly in cages to 15 mg./kg. when the mice were put three to a cage following injection. Pretreatment with 5 mg./kg. of chlorpromazine increased the LD_{50} of amphetamine in single mice from 111 mg./kg. to 144 mg./kg., but increased the LD_{50} of grouped mice from 15 mg./kg. to 121 mg./kg. (80). Promazine and reserpine had similar protective actions, and so did phenobarbital, but only in doses causing ataxia. Interestingly, pentobarbital was without protective action. Essentially similar results have been obtained, apparently independently, by Burn & Hobbs (20) for reserpine and CPZ. These authors found that meprobamate and benactyzine were essentially devoid of protective actions against the toxicity of amphetamine in grouped mice.

This protective action of CPZ has been independently confirmed a number of times. For example, Nielsen & Neuhold (94) found that 1 mg./kg. of CPZ increased the LD_{50} of amphetamine from about 18 mg./kg. to about 110 mg./kg. (as estimated by the reviewers from the graphs presented by the authors). These authors also found chlorprothixene, a related compound, to have a protective action. Similar results have been reported for CPZ and thioridazine by Swinyard *et al.* (130), the latter drug being considerably less potent on a weight for weight basis. Comparisons have been made of CPZ and methoxypromazine by Gray *et al.* (53), and of CPZ and triflupromazine by Piala *et al.* (106); these three drugs are of approximately the same potency. The last authors introduced the additional feature of mechanical agitation of the grouped mice by putting them in a rotating cylinder, so that even when most members of the group had died, the survivors were subjected to continuing strong external stimulation.

What can be said about this protective action of CPZ and related compounds? Clearly, mice put together in groups after injection with large doses of amphetamine tend to induce in one another behavior which is prejudicial to their survival. Chlorpromazine appears to reduce the responsiveness of the individual mouse to the stimuli engendered by other mice. Rather than antagonizing "directly" the lethal actions of ampheta-

mine, CPZ protects the mice from the lethality-enhancing attributes of the environment. This ability of CPZ and related drugs to reduce the efficacy of stimuli in controlling and directing behavior seems to be an important property of this type of drug, as will become apparent in succeeding pages; it may well turn out to be the salient characteristic of major tranquilizers.

Chlorpromazine has been reported to have only slight protective action against the lethal actions of mescaline (109). Unfortunately, no information is given as to whether the mice were grouped or isolated following injection of mescaline. Thiopropazate, perphenazine, prochlorperazine, and promethazine were much more effective than CPZ, whereas promazine and reserpine were of comparable efficacy to CPZ in protecting against the lethal effects of mescaline.

The effects of tranquilizers on convulsive seizures induced by electricity, convulsant drugs, or sound have been the subjects of a number of investigations. Chen *et al.* (23) found that reserpine did not change the intensity of the electrical stimulation that had to be put through the brains of mice to produce in them maximal seizures, but reserpine did antagonize the ability of diphenylhydantoin to raise this threshold. Chlorpromazine has been reported to lower the intensity of alternating current necessary to induce minimal seizures in 50 per cent of mice; promazine, mepazine, and promethazine had similar actions, but trifluoperazine and prochlorperazine did not (132). Essentially no effect of CPZ on electrically induced complete extensor seizures was found by Kopf & Nielsen (74), although mepazine conferred some protection. Swinyard *et al.* (130) found CPZ and thioridazine to be ineffective against electroconvulsive seizures in mice.

No protective action of reserpine, even in doses of 100 mg./kg., against drug-induced convulsions was found by Tripod *et al.* (135); and Chen *et al.* (23) have found that reserpine reduces the dose of pentylenetetrazol and of caffeine, but not that of strychnine, necessary to cause convulsions. Swinyard *et al.* (130) found neither CPZ nor thioridazine to alter the amount of pentylenetetrazol necessary to cause convulsions.

On the other hand, Tripod *et al.* (135) found that 100 mg./kg. of reserpine could confer some protection against audiogenic convulsions in rats. Using very much smaller doses (0.2 to 1.0 mg./kg.), Bevan & Chinn (6) found that reserpine enhanced the convulsive responses of solitary rats to sound. Even apart from the difference in dosage, these two sets of experiments are hard to compare since few of the experimental circumstances are specified by Tripod *et al.* (135). In experiments on mice, Plotnikoff & Green (108) found that CPZ, mepazine, meprobamate, reserpine, and some other drugs conferred protection against audiogenic seizures, but Fink & Swinyard (47) report no protection against maximal audiogenic seizures with CPZ, promazine, or triflupromazine, except in neurotoxic doses. Neurotoxic doses were defined as doses that prevented mice from staying one minute on a slowly rotating rod, a feat that normal mice can accomplish with careless abandon. Fink & Swinyard found protection against audiogenic convulsions

only in proportion to protection against maximal electroconvulsive seizures. In subsequent experiments in the same laboratory, Swinyard *et al.* (130) have found CPZ and thioridazine to lead to some modification of audiogenic seizures in mice, but not to clear protection in the ordinary sense of the word. The difference in results between Plotnikoff & Green, on the one hand, and Fink & Swinyard, on the other, may be attributable to the circumstances of the tests; Plotnikoff & Green exposed their mice to sound in groups of five while Fink & Swinyard exposed them singly. In view of the results on toxicity of amphetamine, it may be that CPZ and other drugs minimize the tendency of interactions between the mice to enhance the convulsive properties of the sound stimulation without appreciably affecting the action of the sound per se. Plotnikoff & Green do not give any figures for solitary mice, but comment that grouping the mice led them to "seem to stimulate one another; and that thus the observed total response is a group phenomenon." Fink & Swinyard suggest also the possibility of a strain difference in the mice as a contributing factor in the difference in the results.

Another type of unconditioned behavior on which the effects of tranquilizers have been studied is the so-called spontaneous motor activity (SMA) of animals. Spontaneous motor activity refers to that activity the cause of which is unknown or unspecified. Three general techniques have been used for measurement of SMA: "jiggle-cage" devices; "rotating-drum" devices; and "photo-cell" devices. Jiggle cages consist of animal containers suspended in such a way that movements of the animal are transmitted to a displacement recording system. In rotating-drum devices the drum is mounted on a horizontal axle; the animal is free to run inside the drum; running causes the drum to rotate on the axle and these rotations are recorded. Photocell devices have light beams crossing a cage and impinging on a photo cell; animals are put in the cage and each break of the beam is counted. Jiggle cages presumably record all types of movements, whereas the latter two systems record mainly translational locomotion. In practice, the results obtained by the three methods tend to be rather similar. Most studies on SMA have been made in small animals—mice and rats—although use of a photocell device for monkeys has been reported (29).

Chlorpromazine has been reported to reduce SMA of small animals as measured by each of these three types of devices. Kopf & Nielsen (74) employed a jiggle cage using a phonograph pickup transducer; mepazine (74) and chlorprothixene (94) were studied in the same apparatus. The effects of CPZ and perphenazine were studied in a rotating drum by Irwin *et al.* (66). Mirsky *et al.* (91), Gray *et al.* (53), Swinyard *et al.* (130), and Tedeschi *et al.* (133) employed photo-cell devices to study chlorpromazine; meprobamate (91), methoxypromazine (53), thioridazine (130), and trifluoperazine (133) were also studied. In a carefully conducted study, Borsey *et al.* (11a) compared reserpine, deserpidine, CPZ, perphenazine, meprobamate, and trimethoxy-benzoyl-morpholine for their effects on SMA using a photocell device. The Rauwolfia alkaloids were most potent in depressing SMA,

meprobamate least potent, and the phenothiazine compounds intermediate in activity. The increase in SMA caused by amphetamine is antagonized by CPZ and some indolylethypyridine derivatives (91), by thioridazine (131), and by trifluopromazine (the latter as measured in a device counting the number of contacts made by a mouse between the floor of the cage and either the sides or some wires crossing the cage) (106).

Effects on conditioned behavior.—An effect of CPZ on conditioned behavior was described in the first acount of its pharmacology by Courvoisier *et al.* (27). Experiments were conducted on rats in a cage with a grid floor which could be electrified. The animal was put on the floor of the cage, and a tone was sounded for 10 seconds before the grid was electrified. The animal could then escape from contact with the electrified grid by jumping on an insulated pole that descended from the roof the cage. It was found that after a number of trials the rat climbed the pole during the 10 seconds of tone, thereby avoiding the shock. Following appropriate dose of CPZ, the rats failed to climb on the pole during the tone, but still did so when the grid was electrified. The climbing response to the shock was referred to as an unconditioned response (UR), and the climbing response to the tone as a conditioned response (CR); the effects of CPZ were described as abolition of CR while UR remained intact (27). This terminology has been adopted by a number of subsequent workers. It is misleading, unfortunately, since the climbing response to the shock is not an unconditioned response in the proper meaning of the term; that is, it is not a response which occurs on first presentation of the stimulus and in much the same general form in all members of the species. The misunderstanding probably arose because the shock, in contradistinction to the tone, does indeed elicit unconditioned responses; but it does not follow from this that all responses occasioned by the shock are unconditioned. On the contrary, the first time the rat is put in the cage and exposed to the shock, the response consists of "random escape movements" (26)—running around the cage, jumping in the air, sometimes biting the grid, or "freezing." Once the effective escape response is made, it rapidly becomes very consistent and prompt, but the process is clearly one of conditioning. Most rats then gradually develop the avoidance response to the tone. The distinction between response to shock and to tone is therefore not between UR and CR but rather between the tendency of the animal to make a specific discriminated conditioned response to each of two stimuli: one (the shock) which is from the first aversive but which comes to occasion a specific discriminated response (jumping on the pole) and the other (the tone) which is originally more or less neutral, but which comes to function as a conditioned aversive stimulus. An aversive stimulus is a stimulus which will sustain behavior that leads to its cessation or postponement.

This criticism of terminology does not, of course, reflect on the findings of Courvoisier *et al.* (27), which have, in fact, been repeatedly confirmed. Using a similar apparatus, Cook & Weidley (26) found that the dose of

CPZ necessary to cause 50 per cent loss of climbing response to tone (buzzer) was about 10.5 mg./kg. by mouth, but that 40 mg./kg. was required to cause appreciable loss of response to shock; barbital, in contrast, caused about a 40 per cent loss of response to shock at the dose that caused 50 per cent loss of response to tone. Differential loss was obtained with reserpine, but two doses of 25 mg./kg. each were apparently necessary. Methylparafynol and meprobamate produced an equal loss of response to both stimuli. Morphine gave essentially the same results as CPZ, and the effect of pentobarbital was intermediate. In a later contribution from the same laboratory, the ED_{50} for CPZ for loss of response to tone is given as 11.9 mg./kg. (133). Generally similar results have been obtained by other workers for CPZ (73, 106, 130) and for trifluoperazine (133), triflupromazine (106), thioridazine (130), and reserpine (73). Since the absolute values obtained must depend on the details of the apparatus and the exact experimental procedure (see later), no attempt will be made to combine the results from different laboratories.

It is clear that there is a differential sensitivity to suppression, by CPZ and relatives, of the response to the warning stimulus and to the shock; in other words, the avoidance behavior is more readily suppressed than the escape behavior. Avoidance behavior may be defined as behavior that occurs in the presence of originally neutral stimuli, correlated in the past with a succeeding aversive stimulus, such behavior having as its consequence the prevention or postponement of the aversive stimulus. Escape behavior is behavior that occurs in the presence of aversive stimulation and that leads to cessation of the stimulation. The distinction between avoidance and escape behavior as a basis for differential effects of drugs is implicit or explicit in the description of a number of experiments using more conventional psychological techniques, which, in contrast to the pole-climbing technique, do not involve repeated handling of the animal by the experimenter during the experimental session. Handling obviously introduces an uncontrollable and unspecifiable set of variables into the situation. One such technique is the "shuttle box," which is a cage divided into two equal compartments by a hurdle over which an animal can pass. Each compartment has a grid floor that can be electrified. The animal is put in one compartment; a visual or auditory stimulus (discriminative stimulus) is then presented, followed after a predetermined interval by electrification of the grid on the side of the animal. The animal can escape the shock by crossing the hurdle; alternatively it can avoid the shock by crossing the hurdle during the period of warning stimulus. After a predetermined intertrial interval the discriminative stimulus is again presented and the animal must recross the hurdle in the opposite direction to avoid or escape shock. This procedure can be continued indefinitely, the animal shuttling from compartment to compartment. In rats in this situation Nielsen & Neuhold (94) found that 4 mg./kg. of CPZ caused 50 per cent loss of avoidance with essentially no loss of escape, and Irwin *et al.* (66) found 50 per cent loss of avoidance at

1.4 mg./kg. and 50 per cent loss of escape only at 16.7 mg./kg. The comparable figures for perphenazine were 0.1 and 1.2 mg./kg., respectively. Methoxypromazine was found by Gray *et al.* (53) to be approximately equipotent with CPZ and to cause equivalent dissociation of avoidance and escape. Complete loss of both avoidance and escape responding occurred in a cat eight hours after administration of 0.07 mg./kg. of reserpine (67). Reserpine at a dose of 0.37 mg./kg. caused 50 per cent loss of avoidance responding in a monkey (123), and 1 mg./kg. of CPZ caused 100 per cent loss (124). Pentobarbital produced loss of avoidance behavior, possibly as a result of ataxia (124). Complex interactions between Rauwolfia alkaloids and CPZ and brain damage in monkeys in relation to avoidance responding have been reported (123). Brain-damaged monkeys are claimed to be less sensitive to reserpine and desmethoxyreserpine, but not less sensitive to rescinnamine and chlorpromazine. These surprising results have not been independently confirmed.

A still more refined situation for studying escape and avoidance behaviors has been applied by Verhave *et al.* (138) to drug studies. Rats received a shock through the grid floor of the cage unless they rotated a conveniently located wheel within seven seconds of onset of a buzzer. Once the shock had started, it could be terminated by rotation of wheel (escape). Their findings are in general agreement with those obtained with other procedures: 25 mg./kg. secobarbital caused about a 70 per cent loss of avoidance but about a 40 per cent loss of escape, while 4.0 mg./kg. CPZ caused about 80 per cent loss of avoidance and only about 5 per cent loss of escape.

The results just described with CPZ and reserpine and some of the other drugs are compatible with a differential effect of the drugs on avoidance behavior as opposed to escape behavior. They do not, however, serve to establish such a specific effect; other possibilities remain. Some information on this point has come from experiments on the pole-climbing situation. Workers using this technique have commonly observed that, on being put in the apparatus, well-trained rats promptly ascend the pole, even before the auditory stimulus is presented. This has been the subject of explicit study by Maffii (84) under the title of "Secondary Conditioned Response." He found that the ED_{50} of CPZ for preventing the climbing response before the auditory stimulus was 1.75 mg./kg.; the ED_{50} for preventing the climbing response during the auditory stimulus was 11.6 mg./kg.; and that the ED_{50} for preventing the climbing response when the shock was presented was 33 mg./kg. Thus there is a differential sensitivity to CPZ of the tendency to respond to the "environment of the box" and of the tendency to respond to the environment plus auditory stimulus, just as there is a differential sensitivity to CPZ of the tendency to respond to the tone and of the tendency to respond to the shock. This suggests that there may be a continuum of peremptoriness of stimuli in occasioning the response, that in this situation, shock > auditory stimulus > "box alone." If the effect

of CPZ were to attenuate the power of the stimuli to occasion the response, then it would be expected, as found, that the effects of CPZ would be seen first (in the sense of in lowest dosage) on the response to the stimulus of least efficacy. This concept has the advantage of much wider applicability than that which supposes the differential effects of CPZ to be based on a dichotomous distinction between avoidance and escape behavior; and, as will be indicated below, there are indications that this sort of an effect of CPZ occurs in situations where there are no aversive stimuli and, hence, no escape or avoidance behaviors in the present usages of the words. Maffii (84) studied a number of other drugs. Promazine, reserpine, and morphine gave differential effects similar to CPZ. However, meprobamate, hydroxyzine, azacyclonol, phenaglycodol, and phenobarbital abolished the response to the auditory stimulus only at doses very close to those abolishing responses to the shock, although the pre-buzzer responses were abolished at about one-third or less of this dose level. A different approach along the same lines has been reported by Gatti (50, 51). An analytical approach of this kind is obviously the right way to find out more about the drugs and the differences between them. It may be questioned, however, whether the pole-climbing situation is the best system for analysis of this type of phenomenon.

Other attempts have been made to account for the differential effects of CPZ or reserpine on different behaviors. Examples which will be discussed are that CPZ has differential effects depending on the type of motivation maintaining the behavior (e.g., aversive vs. positive reinforcement), on learning as opposed to performance, or on "emotional" behavior. A fundamental difficulty in establishing such distinctions as the basis of differential effects arises out of the finding that differential effects of CPZ can be seen on behaviors which have the same motivation, comprise the same response, and occur in the same animal during successive short periods of time; and which differ only as a result of certain procedural differences, that might, at first glance, appear to be trivial. These procedural differences relate to the programming of the temporal and sequential relations between stimuli presented to an animal, responses of the animal, and further stimuli consequent upon those responses. The first and last of this series of events are prescribed by a "schedule." These are not trivial matters; on the contrary, the importance attributed to schedules in determining behavior has been growing steadily since the days of Pavlov, and they are now beginning to overshadow the traditional variables—motivations, emotions, etc.—that, ever since the Greeks formulated them, have been supposed to be the essential and only determinants of behavior. The relevance of this for the present context is that it has been shown repeatedly, as will now be exemplified, that the behavioral effects of a drug are frequently critically dependent on schedule influences on behavior (see, for example, 32, 34, 38, 118). When attempts are made to compare the effects of drugs on, for example, behavior maintained by aversive stimuli as opposed to behavior maintained by food, it usually happens that, inadvertently or inevitably, the schedule is

different in the two situations. In such cases it is gratuitous to assume, in the absence of further information, that differential effects of the drug are based on motivational differences rather than on schedule differences. Similar remarks apply to most studies on learning and emotion. These basic considerations are still not usually appreciated. In the great majority of published studies such additional information is lacking. An extremely clear statement of some of the difficulties of establishing the specificity of a drug effect is given by Weiskrantz (141). Briefly, study at several values of the independent variables of the behavioral situation (so-called parametric studies) of more than one drug, each at a series of dose levels, is the minimum program before claims as to specificity of a drug effect can be seriously considered. It is recognized that this represents a prodigious amount of work; but when dealing with a subject matter as complex as behavior, surely inferences should be made with more than usual conservatism, rather than, as has so often been the case, just the opposite.

Dependence of drug effects on schedule considerations can be recognized in a great many descriptions of drug effects in the literature. It was first demonstrated explicitly, in pigeons, for pentobarbital (32). As regards CPZ, experiments on pigeons working in a positive reinforcement situation have led to the suggestion that one aspect of its effects is an attenuation of the power of stimuli, internal or external to the animal, to occasion the occurrence (or nonoccurrence) of the responses (8, 35, 36). This interpretation is, of course, similar to that advanced to account for the dissociation between escape and avoidance behavior by CPZ in the experiments described above, and indicates the generality of its applicability.

The effects of CPZ on performance of rats working under a multiple schedule of reinforcement have been reported by Weissman (146). (A multiple schedule is one in which two or more different schedules of reinforcement are imposed during each session, each correlated with a distinctive, different exteroceptive stimulus.) The effects were compared with those of morphine, amphetamine, pentobarbital, iproniazid, and nialamide. There were indications of differential effects both between the different components of the schedule and between the drugs.

Experiments have been made on the effects of CPZ, reserpine, meprobamate, and phenobarbital on rats in a Lashley jumping-stand apparatus (45, 82). In this procedure a rat is put on a small grid platform facing two windows but separated from them by a chasm across which the rat must jump. If the jump is towards the "correct" side, the window opens and the rat passes through to food; if toward the "wrong" side, the window does not yield, and after bumping its nose on the window the rat falls some distance into a net. In each trial one or the other of the windows is lighted. The gird of the platform is electrified some predetermined time (e.g., 30 seconds) after the rat is placed on it, so that it receives a shock if it has not already jumped. The situation is obviously complex, involving shock avoidance, food on a "correct" response, and punishment on a "wrong"

response; also "sidedness" and a visual discrimination. The clearest effect of CPZ, reserpine, and meprobamate was to increase the time before jumping so that the treated animal regularly received a shock before jumping; untreated animals did not. This effect is reminiscent of the effects in the pole-climbing situation. "Fixated" behavior (in the sense of producing an animal that jumps to the same side each time) can be developed in the Lashley apparatus by making neither side nor lighting of the window indicative of the "correct" side. Chlorpromazine and reserpine did not specifically annul this fixation at the doses used (82). It was also shown that CPZ, at the single dose studied, did not affect a brightness discrimination function (45). On the other hand, when fixated behavior was produced in a T-maze by making each arm equally likely to yield food or shock (under which schedule the rats went consistently to one side) the fixated behavior was modified by CPZ, since some six of 14 rats changed side with the dose selected (93). The complexities of these situations defy analytical comparisons at the present time.

A situation in which CPZ and pentobarbital had effects in opposite directions has been described by Blough (9). In these experiments, a pigeon obtained food by blocking both of two horizontal light beams crossing its cage at right angles at head height by keeping its head in the area of intersection of the beams. Each time food was obtained, the time that the beams had to be blocked for the next food presentation was increased by two seconds; concurrently and independently, the time necessary was reduced by two seconds every four minutes. This procedure led to stabilization of rates of reinforcement of about one per four minutes. The average required response time was used as the measure of the performance. From a control level of about five seconds this time increased to about 10 seconds following 10 mg./kg. of CPZ and decreased to less than two seconds following 10 mg./kg. of pentobarbital. Dose-effect curves were obtained permitting the specificity of the difference in the effects of two drugs on the particular behavioral performance to be established.

Effects on motivation.—Chlorpromazine caused a dose-dependent reduction in the food intake of rats during a one-hour period concluding 23 hours without food and a reduction in water intake under similar deprivation conditions (115). The dose levels used, however, are known to modify a variety of behaviors, and no evidence of the specificity of depression of consumatory behavior is given. Chlorpromazine has been reported to depress sexual behavior in male rats (149). Chronic dosage with 0.075 mg./kg. of reserpine depressed water intake by rats (140).

The ability of electric shocks delivered to certain parts of the brain (intracranial self-stimulation) to maintain behavior which has as its only programmed consequence the delivery of such shocks has been amply substantiated since its original description by Olds & Milner (100). The effects of CPZ and reserpine on this type of behavior have been described as differing, depending on the position of the electrode (98). Both drugs caused

marked depression of responding when the electrode was in the hypothalamus or amygdala, but only minor depression when the electrode was in the septal region. A later publication (99) described similar results for CPZ but not for reserpine. A still later publication by Olds & Travis (101) indicated that CPZ caused a large decrement in responding even when the electrode was in the septum. Olds (97) has also suggested a differential effect of a number of drugs on "positive" and "negative" reinforcing areas of the brain. The drugs fell into two groups:

> those effective against escape in doses that fail against self-stimulation—pentobarbital, morphine, and meprobamate—and those effective against self-stimulation in doses that fail against escape—chlorpromazine, and on the basis of earlier work, probably reserpine.

Olds concluded that "every chemical which inhibits approach mechanisms fails to inhibit escape mechanisms" and vice versa. Some of the difficulties in reaching conclusions of this kind have been mentioned already. In addition, the earlier work by the same author (98, 99) showing differential sensitivity for different "positive" areas of brain to CPZ would seem to make this suggestion untenable. The paper by Olds & Travis (101) also reported extensive experiments on brain mapping using CPZ, morphine, meprobamate, and pentobarbital. The reviewers have the impression that some of the other results described in this paragraph are rescinded by this extensive report, but they are not quite sure.

Other effects of CPZ on intracranial self-stimulation have been reported by Stein & Ray (126). In their experiments, rats were presented with two levers. Depression of one lever led to stimulation in either the posterior thalamus or the midbrain tegmentum; the shock intensity decreased progressively with each lever press, but could be reset to its original level by a single operation of the other lever. At a dose level of 1.5 mg./kg. of CPZ the animal pressed the reset lever after fewer consecutive presses of the primary reinforcing lever; it also pressed the primary reinforcing lever more frequently. This latter increase in rate was postulated to result from an effect of CPZ in shortening the aftereffects of the brain shock, but no direct evidence was presented. What was observed was increased rate of responding on both levers; there are many other situations, not involving brain shocks, in which CPZ also causes an increase in rate of responding.

A direct study of type of motivation as a possible basis of differential effects of reserpine has been made by Wenzel (147). Eight cats were trained to press one lever to obtain food in the presence of one stimulus and to press a second lever in the presence of a second stimulus to avoid or escape from electric shock. The cats were then given 35 μg./kg. of reserpine and tested repeatedly in the succeeding 124 hours. The median latencies of the responses to the stimulus for food were increased much less than those to the stimulus for shock. It was concluded that the type of reinforcement played a crucial role in determining the susceptibility of the responses to

the effects of reserpine. This conclusion is in contrast to the equivocal or even contrary findings on this point by other workers (see below); it therefore deserves careful evaluation. Wenzel's figures show that the prereserpine latencies for the avoidance response were, on the average, longer than the latencies for the food response (medians of four seconds and two seconds, respectively). If the latency, the only measure used in this study, is to be taken seriously as a quantitative measure, this difference cannot be dismissed. Immediately before reserpine, three out of the eight animals showed lower or equal median latencies to the preshock stimulus than to the prefood stimulus; after reserpine, in the 96 series of trials conducted, 22 still showed median latencies to the preshock stimulus equal or lower than those to the prefood stimulus. Considered this way, this is not very impressive evidence for specific reduction in shock motivated behavior. The statistical significance depends, presumably, on about one-half the cats that gave very high latencies for shock, in one or two series, at irregular intervals after the drug. The avoidance response showed greater resistance to extinction than did the food response, but this is not conclusive evidence for the greater "strength" of the avoidance response at the beginning of extinction, since in extinction of the food response the absence of reinforcement is apparent at every response, whereas in extinction of the avoidance response the lack of correlation between absence of shock and occurrence of responses is much less obvious. Another difficulty in interpreting the results arises from the earlier observations in the same laboratory (69) that reserpine caused a very similar dissociation in effects on latencies of response to an auditory and to a visual stimulus, both of which were occasioning an avoidance response; the latencies to the visual stimulus increased while the latencies to the auditory stimulus remain unchanged. The reserpine in these experiments was given into the cerebral ventricle in a dose of 200 μg., but, nevertheless, the experiments serve to show that the dissociation seen between avoidance and food response latencies can also be seen in a differential effect on the stimulus control of an auditory stimulus vs. a visual stimulus. The authors conclude that the differential susceptibility "is related to the difficulty in learning the two responses, for avoidance to tone was acquired in fewer trials. . . ." Thus the difference in the ability of different sensory modalities to pre-empt the behavior of the animal is as able as differences in motivation to lead to differential effects of reserpine. It is interesting that in these experiments, as in Wenzel's, the stimulus whose control was most markedly affected by reserpine was that to which the initial latency was the longer. It seems possible that all these differential effects of reserpine will turn out to be based on a continuum of strength of stimulus control of behavior as suggested earlier in this chapter for CPZ.

In contrast to the conclusions of Wenzel (147), Miller (86), Riopelle & Pfeiffer (112), and Weiskrantz & Wilson (142) found no evidence of greater sensitivity to reserpine of avoidance behavior than of positively reinforced behavior in monkeys, and Sidman (118) concludes that "the

nice classification that seemed to be developing for reserpine on the basis of positive- and negative-reinforcement contingencies did not hold." For CPZ, the converse hypothesis, that behavior maintained by positive reinforcement is more sensitive than that maintained by aversive stimulation, has been proposed by Olds (97), and Ferster & Skinner (46) show evidence pointing in the same direction. In Weissman's (146) multiple-schedule experiment, CPZ appeared to have rather more effect on shock-avoidance behavior than on food-maintained behavior.

In conclusion, it cannot be regarded as established that reserpine and CPZ have a greater effect on behavior related to aversive events than they do on behavior related to positive reinforcement. Researchers have been predisposed to look for differential effects of these drugs along these lines because of the general impression that they are useful clinically, especially in reducing the reactions to aversive stimuli and situations. It must be remembered, however, that few people consult physicians because their work has too much fascination for them, or because they enjoy their play too much; the people to whom the drugs are given most frequently are by no means a representative sample of the general population. The drugs might be just as effective in alleviating fascination and enjoyment. Indeed, one of the most dramatic therapeutic effects of the drugs is in quieting manic psychotics in most of whom there is no reason to infer aversive stimulation as the cause of hyperactivity.

Effects on acquisition and extinction.—Several attempts have been made to assess the effects of major tranquilizers on acquisition or extinction of behavior. Since these drugs cause such profound changes in performance, it becomes a matter of considerable difficulty to identify specific effects on changing performance. For example, although it has been shown that rats require significantly more trials to reach a criterion of 14 out of 15 avoidance responses in a shuttle box and also that these responses are extinguished more rapidly when under CPZ (1), these effects, as the authors point out, could be caused by "motor retardation," rather than effects on acquisition or extinction. They could also be caused by effects on stimulus control. Comparable difficulties apply to the interpretation of the semiobjective study of CPZ by Denenberg *et al.* (31), and no conclusions on the effects of reserpine in this regard can be drawn from the single, ineffective dose study of Wayner & Reimanis (140). Miller *et al.* (89) found that CPZ produced a dose-dependent decrease in the number of responses of rats during extinction in a shuttle box. Another study (90) reported more rapid extinction of a shuttle-box avoidance response in rats under CPZ than under saline and extinction persisted when the CPZ was discontinued. Although the CPZ clearly had an effect during extinction, this does not constitute proof that CPZ has an effect on the process of extinction. For example, prolongation of latencies under CPZ (an effect on performance) would lead from the beginning to more occasions in which the buzzer was terminated without a shock, which would surely speed the progress of

extinction. This may be what the authors have in mind when they suggest that their findings "were attributable to relearning of the avoidance situation by animals during administration of CPZ." A comparable explanation is suggested by Hunt (65) to account for his observation, under entirely different circumstances, of a failure of extinction under CPZ. Thiopropazate has been shown to depress both escape and avoidance behavior by rats during acquisition of the responses in a discrete, lever-pressing situation (128). Comparisons were made, at a single dose level, with benactyzine and pipradol.

Effects on conditioned suppression.—A great deal of interest has been directed toward the possibility that tranquilizers specifically modify "fear" or "anxiety," probably because of the general impression as to the clinical usefulness of the drugs. Experiments suggesting such an effect of reserpine have been described several times by Brady (15 to 18) using the procedure of Estes & Skinner (43). Rats or monkeys were trained to press a lever for positive reinforcement. While this lever-pressing behavior was continuing, a "neutral" stimulus (i.e., a stimulus which on initial presentation had little or no effect on behavior), such as a clicking noise, was presented for a period of time, ending coincidentally with delivery of an electrical shock to the animal; the shock was delivered irrespective of the behavior of the animal. If the durations of the "clicker-on" and the "clicker-off" periods are suitably chosen (127), the animal comes to respond at a very much lower rate during the clicker-on periods (conditioned suppression). After some days on a daily reserpine dose of 0.20 mg./kg. to rats or 0.75 mg./kg. to monkeys, the response rate during the clicker periods increased and the rate when the clicker was not on decreased so that the two rates became much more nearly alike. When the rate during the clicker period was reduced by making every response (later every second or third response) lead to a shock (i.e., a punishment situation), the rate did not increase under the influence of reserpine. This was interpreted as evidence of a relatively specific effect of reserpine on "anxiety" (15). There is no published independent confirmation of these findings. The possibility remains that the effects of reserpine could all be attributable to attenuation of the control of the behavior by the clicker. The rates during the clicker were much lower on the punishment procedure than on the conditioned suppression procedure indicating that the control of behavior by the clicker was more powerful in the former instance, and such differences in degree of stimulus control have been shown to be adequate to cause differences in susceptibility to disruption by drugs (33). On the other hand, evidence of specificity of the effects of reserpine is provided by comparison with amphetamine, which caused an increase in rate during the clicker-off periods with as great or greater than normal suppression during the clicker periods. Unfortunately, no information on dose-effect relationships is given for either drug. In rats in a comparable situation, except that the response was a lick of a water bottle by the rat, it has been shown that while small doses of ampheta-

mine did not attenuate the suppression, larger doses led to rapid responding through the stimulus period (134).

Description of this effect of reserpine on conditioned suppression in terms of anxiety conforms with contemporary psychological usage, but it is probable that it has led to interest in these findings in circles to which they would not otherwise have penetrated and has contributed to confusion among the psychologically unsophisticated. There appears to be no generally accepted definition of anxiety. Clinically, anxiety is usually characterized on the basis of the verbal behavior of patients; for example, Persky *et al.* (105) write that in their studies on anxiety "the criterion of anxiety was the patient's experience of a feeling of dread and foreboding—as if something dangerous were about to happen." To a large school of psychologists, anxiety is a drive—something that will sustain escape or avoidance behavior (87, 92). Again, other psychologists emphasize as a defining characteristic of an emotion such as anxiety that it affects a number of different behavioral activities simultaneously and nonspecifically (43). It is mainly in this last sense that the term is used by Brady, although in his experiments subjective assessment was also made of defecation, pilo-erection, and immobility. It does not seem to the reviewers that there is any question of "right" and "wrong" usage here, but merely one of convention. Of course, there has been a vast amount of illuminating work on the relations between the different "aspects" of anxiety, but it is still not possible to use the term interchangeably to mean any or all of these aspects. Attempts to do so lead to sterile arguments as to the applicability of findings in animals to man, and distract from the careful comparison of specific phenomena which is the only sound basis for such inferences.

Using a subjective assessment of a conditioned emotional response, Weiskrantz & Wilson (143) studied the effect of reserpine in monkeys; no validation of the scheme is given. On the basis of these observations, they suggest that reserpine interferes with the development of a learned emotional response. This suggestion has been challenged by Stein (125), who studied the development of conditioned suppression under reserpine. The animals were maintained under reserpine during the pairings of periods of clicking noise and shock. They were then taken off the reserpine and their performance during extinction (i.e., during continued presentations of the clicking noise but without shock accompanying its cessation) was compared with that of animals that had been similarly conditioned in the absence of reserpine. Although the dosage of reserpine used (0.5 mg./kg./day) was sufficient to reduce the rats to virtual inactivity by the end of the treatment period, they subsequently showed essentially as much suppression during the clicking periods in extinction as did the animals that had never received reserpine. Stein does not discuss the relation of his findings to those of Brady. Although there is no logical contradiction between them, the results of Stein should at least raise the possibility that reserpine does not affect fear as such, and that an alternative explanation for the results

of Brady should be sought. In addition to the ever present possibility of an effect on stimulus control, Heistad (61) has suggested an attenuation of conditioned suppression based on discriminable modification of the internal environment of the animal by the drugs; unfortunately, his experiments gave equivocal results.

The effect of reserpine on conditioned suppression in the guinea pig has been studied by Valenstein (136). Such suppression could be produced in the guinea pig as in the rat and monkey, although with the same schedule as had been used in rats, it was less complete. The effect of reserpine, however, was quite different in that it apparently never led to the type of effect reported in rats and monkeys. Reserpine caused either virtually complete abolition of responding only during the clicker periods, or, with larger doses, it abolished responding in the absence of the clicker periods while bursts of responding continued during the clicker periods. The difference between the results in rats and monkeys and in guinea pigs is attributed to a species difference, but it is clear that these fascinating but complex phenomena deserve further analysis. The tendency of the guinea pig to respond is reduced by doses of reserpine on the order of 100 times less than those required in the rat or monkey.

Effects observed by inspection.—The most immediately obvious way of studying the effect of a drug on the behavior of an animal is to give a dose of the drug and then to watch the animal. This has the advantage of simplicity so far as apparatus is concerned; but the apparatus is the only simple thing about this seductively simple-sounding common-sense approach. There are two major difficulties to such "straight forward" observational procedures: the difficulty of obtaining data in reproducible form—that is, reproducible by other people in other laboratories—and the difficulty of summarizing information on dose-effect relationships so that evidence of drug specificity can be substantiated. The former difficulty necessitates the development of relatively elaborate scoring systems, rigid definition of criteria of different types of behavior, use of blind techniques and statistical validation; these are by no means simple undertakings. It should be noted that many of the difficulties of these techniques are avoided when they are used, so to speak, intramurally, by one or a very few observers constantly involved, as, for example, in screening substances in an industrial laboratory. In these particular circumstances simple observational techniques have unquestionably proved their usefulness. The challenge is to convert these findings into permanent contributions in the scientific literature that can be independently verified in any place at any time. Schemes attempting to make this possible have been published for a number of species recently. Two of the most careful scoring schemes have been selected for discussion.

The first is for cats (96). This defines 20 behavioral activities of the species; these are grouped into four categories, each containing five components. The categories are entitled "hostility," "excitement," "contentment," and "sociability." The scheme was subjected to statistical analysis,

and an ingenious attempt was made to enhance the discriminatory power by giving greater weight to the components that occurred least frequently under control conditions. Some six drugs were studied, including CPZ and Rauwolfia. Chlorpromazine caused the least reduction in sociability of any of the drugs and the greatest reduction in hostility, and an increase in excitement. Rauwolfia, on the other hand, caused only a slight drop in contentment along with a drop in sociability, with no change in other categories.

Another scheme, applicable to cats, dogs, and monkeys, has been outlined (66). This also has some 20 items including "curiosity," "playfulness," and "aggressiveness." The complete scheme and its statistical validation have not yet been published. It is reported that the most consistent gross behavioral effect of CPZ was

> suppression of locomotor activity, and, where present, a reduction of fearfulness. A diminution of play and grooming activity and of fearfulness were among the earliest behavioral changes observed. Increased vocalization (meowing) in the cat also was observed with relatively small doses of drug. . . . Aggressive (biting, attacking) behavior of hostile animals usually was suppressed by small doses. . . . In less aggressive animals, however, aggressive behavior often was augmented by the drug(s).

A limitation of these observational schemes is illustrated by the fact that the reviewers are unable to decide to what extent this author agrees or disagrees with the previous author, who found CPZ to lead to "least reduction of Sociability, greatest reduction in Hostility, and an increase in Excitement." Again, neither author presents dose-effect relationships. Although the anthropomorphic terms in these schemes can be given careful definitions, it is somewhat to be feared that with the passage of time the careful definitions will be forgotten and the terms assumed to have their everyday or psychiatric meanings. It is perhaps worthy of note that the reviewers find little evidence in these descriptions of a specific action of CPZ on anxiety, although it is probable that the observers were alert to the possibility of such effects.

A much simpler observational scheme in mice and rats has been used to compare the potencies of reserpine with closely related substances and with whole root preparations of Rauwolfia (114). By this test, 11-desemethoxy-reserpine was equipotent with reserpine, but rescinnamine was much less potent, although in dogs rescinnamine was at least as potent as reserpine on blood pressure, on "sedation" (rated on a scale of one through four), and on miosis.

Observations in man.—Most of the observations on CPZ and related drugs on normal human subjects have involved psychological assessment by means of a "battery of tests"; that is to say, a series of tests intended to measure a variety of aspects of human performance, e.g., simple motor functions, complex motor functions, discriminatory behavior, learning, etc. The first such study was by Heimann & Witt (59), who obtained effects with 37.5 mg. of

CPZ, total dose, intramuscularly. Subsequent studies have usually used larger doses by mouth. Doses of 100 mg., 200 mg., and 400 mg. of CPZ by mouth were used by Kornetsky *et al.* (76), and the effects on performance on a battery of tests compared with those of secobarbital, meperidine, and *N, N*-diethyl-D-lysergamide (LSD). In later experiments using a modified battery of tests, Kornetsky & Humphries (75) showed significant differences from the placebo of 100 mg. and 200 mg. doses of CPZ. Two-hundred mg. of CPZ had a greater effect on motor co-ordination but a lesser effect on "intellectual functioning" than did 200 mg. secobarbital. Surprisingly, this dose of CPZ had a greater tendency to cause sleep in the subjects than did the same dose of secobarbital. This was also seen in a further study in which CPZ was found to have more effect than secobarbital in hastening onset of sleep when given at 7:00 p.m.; both drugs tended to cause "hangover" in the sense of impairment of performance on the test battery next morning between 9:00 and 10:00 a.m. (79). After single doses, 200 mg. CPZ causes more impairment than 200 mg. secobarbital in schizophrenics, but after 11 days of daily treatment with the same drug, 200 mg. CPZ no longer causes significant impairment, although 200 mg. secobarbital still does (78).

Miscellaneous.—Tolerance to CPZ has been studied in rats by Boyd (12). Chlorpromazine reduced spontaneous motor activity as measured in a revolving drum, but after daily dosing the effect was no longer seen, although it could be reinstated by an increment in dosage. Following abrupt withdrawal of CPZ after 40 weeks continuous treatment with doses that towards the end were as large as 200 mg./kg., a large increase in SMA resulted which was apparently sustained for weeks.

Ethiazine (N-2-dimethylaminoethyl phenothiazine; i.e., promazine with a two instead of a three carbon chain between nucleus and side chain nitrogen) has been studied for effects on a Pavlovian conditioned response (salivation) by Aganyants (2). It led to some increase in salivation by dogs to both "positive" and "negative" conditioned stimuli but to complete loss of a delayed reflex. There was loss of inhibition of respiration on presenting ammonia in inspired air, and an increase, instead of the usual conditioned decrease, in respiration during presentation of a stimulus succeeded on previous occasions by ammonia in inspired air. Although it is difficult to make a detailed comparison of these results with those obtained on CPZ previously described, the reviewers feel that they are generally concordant.

Raunescine and iso-raunescine have been reported to have effects qualitatively similar to reserpine on operant behavior in pigeons, although only, respectively, about 1/10 and 1/100 as active on a mg. per mg. basis (102).

Some benzoquinolizine derivatives have been reported by Pletscher *et al.* (107) to be two to five times less potent than reserpine on avoidance and escape behavior in a pole-climbing situation. It is interesting to note that iproniazid, dopamine, norepinephrine, epinephrine, serotonin, and morphine also abolished avoidance behavior before escape behavior; this leads to some

question as to the specificity of the effect. Another benzoquinolizine, tetrabenazine, has been shown by Heise & Boff (60) to produce loss of continuous avoidance in rats in a lever situation during the period between one-half and four hours following a 2 mg./kg. subcutaneous dose. This suppression of avoidance responding by tetrabenzine is antagonized by "monoamine-oxidase inhibitors" in a nicely dose-dependent manner, so that the situation has been proposed as a method of assay for this type of activity of "monoamine-oxidase inhibitors."

Morphine.—Morphine has been studied in a discrete avoidance situation. In discrete avoidance a warning stimulus is presented to the animal; if the animal does not make the required response within a specified period of time, an aversive stimulus supervenes. If the animal makes the required response soon enough, then the warning stimulus ceases and no aversive stimulation occurs. In either case, there is a period of dead time before the warning stimulus is next presented. The dependent variables are usually proportion of trials on which avoidance occurs and latency of the response. This type of situation is frequently referred to in the literature as "classical" or "Warner" avoidance. It is to be distinguished from the situation in which the animal receives shocks not preceded by a warning stimulus at regular intervals throughout the experiment unless responses occur; each response postpones the shock for a specified period. The dependent variables are usually rate of responding and number of shocks received. This situation will be referred to as continuous or "Sidman" avoidance.

The effects of morphine on discrete avoidance in rats with a lever press as the required response has been studied by Verhave *et al.* (139). The ED_{50} for loss of avoidance responding was of the order of 10 mg./kg., at which dose there was essentially no loss of escape responding.

Nalorphine (1 mg./kg.) has been shown by Weiss (144) to depress the rates of responding of rats working for food and to change the temporal distribution of the responding. Mixtures of morphine and nalorphine were also studied.

Morphine has been shown to cause a dose-dependent attenuation of conditioned suppression in rats (63). At a dose of 9 mg./kg. the rate increased approximately threefold during the preshock auditory stimulus while the rate in the absence of the auditory stimulus fell by about 25 per cent. The net effect was thus to make the rates more similar either in the presence of, or in the absence of, the auditory stimulus: i.e., to reduce the stimulus control of the auditory stimulus. As in the case of reserpine, whether there is an additional effect on the specific etiological factors of the suppression remains to be established.

These same authors studied the effect of motivation on the modification of behavior caused by morphine and pentobarbital in human subjects (62). Motivation was varied by varying the conditions under which morphine was given to addicts; standard incentive was to give a constant dose of morphine after experimental sessions, irrespective of performance; high incentive

involved making the dose of morphine dependent on the performance; for low incentive, the subjects received their morphine at least one week before the experiment and in constant dosage. The dependent variable was the average reaction time to visual stimuli. Fifteen milligrams of morphine or 250 mg. of pentobarbital were injected 50 minutes before the session. The effects of pentobarbital were changed from depressant (tending to lengthen reaction times) to stimulant (tending to shorten reaction times) when conditions were changed from low to high incentive, while the converse change took place in response to morphine. Although this type of pronounced dependence of drug effect on identifiable variables has now been shown a number of times in animal experiments, this study is of great importance as one of the very few in which a comparable analysis has been achieved in man.

Amphetamines and Compounds with Similar Behavioral Effects (81)

As mentioned in the opening section on the tranquilizers, the LD_{50} of amphetamines and related compounds is greatly dependent on environmental circumstances. This was first reported by Gunn & Gurd (56) and has subsequently been studied by Chance (21, 22) and by Höhn & Lasagna (64). The aggregation of several mice in a cage after administration of amphetamine reduced the LD_{50} to of the order of one-tenth of the dose necessary for mice in individual cages. Ambient temperature, size of cage, state of hydration, and ambient noise also had effects, although smaller (21, 22). Strain of mouse is also probably important. The administration of shocks to single rats enhanced the toxicity of amphetamine (145). In view of these gross effects of environmental influences on life and death after amphetamine, it is not surprising that environmental influences should greatly affect the more repeatable behavioral consequences of administration of amphetamine.

Amphetamine decreases the intake of food in a free feeding situation (120) and the intake of water in a free drinking situation (3). Different rates of eating were engendered in rats by permitting some animals access to food only during a two-hour period each 24 hours, while others had free access to food all through the 24 hours. The effects of amphetamine were not affected by differences in rate of eating, but they were shown to be dependent on dosage (120). The suppressant effects of amphetamine on rate of eating are exaggerated in rats taking in abnormally large quantities of food or water as a result of appropriate hypothalamic lesions (42). Ephedrine had similar effects and so did phenylpropanolamine, but epinephrine did not. The increased sensitivity of hypothalamic hyperphagic rats to the effects of amphetamine in reducing food intake has been confirmed by Reynolds (110).

The effects of amphetamine on hunger and thirst have been studied by Miller and his colleagues in rats (86). The dependence of hunger on number of hours of deprivation was studied using a variety of "measures of

hunger": volume of milk drunk, rate of bar pressing for food, the maximum concentration of quinine in milk that the rat would drink, and intensity of stomach contractions. Thirst was similarly "measured" in the hours following administration of 5 cc. 2 molar NaCl. The functional dependences found differed radically according to which method of measurement was used, and so did the effects of amphetamine. No method is given for combining these conflicting results to obtain a clear "measure" of the effect of amphetamine on hunger or thirst per se. On the contrary, the author writes, "We are forced to re-examine and perhaps abandon common-sense categories of generalization according to convenient words existing in the English language." But the author seems reluctant to abandon the terms hunger and thirst, even in face of the type of evidence just quoted. It should be emphasized that the "measures" are in conflict only in so far as they are regarded as measures of the hypothetical unitary variables hunger or thirst; otherwise they simply give valuable information on interesting situations. It may be questioned whether the continued use of terms such as hunger and thirst, fear and conflict can do anything other than hinder the search for scientifically defensible categories of generalization.

Amphetamine, but not metrazole, has been reported to antagonize some of the effects of thiopental on a "conditioned reflex" in rabbits; both drugs antagonized hexenal (95). Some iminazoledicarboxylic acid derivatives have been reported to have similar effects in rats (11).

Amphetamine has been shown to increase the output of avoidance behavior in a variety of situations. Methamphetamine led to an increase in the rate of responding of a rat pressing a lever to postpone a shock in a continuous avoidance situation (137). Amphetamine also increased the output of behavior of rats turning off a very loud noise by pressing a lever (57). In some of these experiments, the noise ceased while a lever was depressed but began again when the lever was released. Following 2 mg./kg. of amphetamine, both the rate of pressing the lever (defined as the number of times the lever was pressed divided by the time the noise was on) and the rate of releasing the lever (defined as the number of releases divided by the time the noise was not on) were increased. This is what would be expected if amphetamine nonspecifically increased the tendency to respond; it is against the suggestion that amphetamine specifically enhances the effects of aversive stimuli.

In spite of the fact that amphetamine decreases the intake of water in a free-drinking situation (3), it increased the rate of licking from a water bottle and also changed the temporal pattern of licking, when the lick was made the effective avoidance response in a continuous avoidance situation (134). In the rat, increased licking in this way seems to lead inevitably to increased water intake so this technique provides a means of accurately controlling the fluid intake of rats in amounts up to several times the normal intake.

The amphetamines will increase the rate of responding even when the reinforcement is food or water. This has been shown for food by Dews (37) in pigeons and by Teitelbaum & Derks (134) in rats, and for water in rats by Brady (15), Sidman (117), and Stone *et al.* (129). This is one of the clearest examples of the predominance of schedule contingencies over other controlling variables, such as motivation, in determining the modification of behavior by drugs.

Larger doses of the amphetamines frequently lead to reduction in output of behavior (37, 86). This is not surprising in itself, even though a profound reduction in behavior occurs with doses that are very much below lethal levels. What is surprising is that the dosage level at which behavioral output falls below control levels varies considerably according to the schedule of reinforcement even when all other features of the situation are kept constant (37). In addition, there are many behavioral situations in which the amphetamines cause virtually no, or only slight, stimulation. The identification of the features of a situation that determine whether optimum doses of an amphetamine will, or will not, cause a substantial increase in output of behavior is clearly a matter of great importance.

There is a tendency, as with other classes of drugs, to consider first the possibility that the effects of the drug are dependent on nature of motivation—that, for example, drugs may affect behavior maintained by positive reinforcement differently from behavior maintained by use of aversive stimuli such as electric shocks. As has been repeatedly emphasized such differences are hard to establish, especially when it has been shown that the drugs cause differential effects on performance depending on schedule of reinforcement under identical conditions of motivation. The reviewers have the impression that the stimulant effects of the amphetamines continue up to higher dose levels when performance is maintained by aversive stimuli than when it is maintained by positive reinforcement, but this has not been clearly established.

Some progress has been made in identifying other factors determining the effects of amphetamines. From the relative effects on performance on four different schedules of reinforcement of pigeons, it has been suggested that control rate of responding is an important factor, that sustained rates of responding are not susceptible to increase, but that very low rates or intermittent responding are readily increased (37). There is, of course, a limit to this relationship, in that if the tendency to respond is extremely low, then the output of behavior cannot be increased by the amphetamines. This has been shown by Verhave (137) in rats. He obtained very low rates of responding by programming no consequences to the lever presses of untrained rats. Six rats had daily one hour sessions of this kind for 12 days. There was a tendency for the number of responses made in the successive sessions to decrease. In the first session the mean was 15.7 responses per hour and all animals made at least one response during the hour. In the seventh session the mean was 0.8 responses per hour and three of the

six animals did not respond at all. This certainly represents a very low tendency to respond. Before the eighth session, the animals were given 2 mg./kg. of methamphetamine; the mean rate of responding remained 0.8 responses per hour and four of the six animals failed to respond. In other experiments in which the tendency to respond was somewhat higher, the effects of the methamphetamine were unpredictable, sometimes causing a substantial increase and sometimes not. When training in an avoidance situation had led to a substantial tendency to respond, the effects of the amphetamines became consistent in causing an increase in rate (134, 137). In pigeons, it has been shown likewise that methamphetamine does not cause an increase in rate of responding when the tendency to respond is extremely low because of prolonged extinction in the presence of a particular stimulus (33).

The effects of methamphetamine on behavior have been shown to be dependent not only on the nature of the schedule and the amount of training, but also on the parameter value of the schedule (38). Pigeons were studied under conditions of delayed reinforcement of the response, i.e., a period of time had to elapse between occurrence of the response and presentation of the reinforcing stimulus. When the delay time was 10 seconds, methamphetamine had little tendency to cause an increase in rate of responding, but it caused a large increase when the delay was 100 seconds. The effects of pentobarbital were also dependent on the delay value, but opposite to those of methamphetamine, since pentobarbital caused a large increase in rate of responding when the delay was 10 seconds but had essentially no effect, on the average, when the delay was 100 seconds.

All these findings are compatible with the view that an important determinant of the effects of the amphetamines is the control rate of responding per se, irrespective of species, or type of motivation, or response studied.

A more or less specific effect of amphetamine on the response of an animal to electric shock has been suggested by Teitelbaum & Derks (134). Superimposed on an intermittent schedule of positive reinforcement for food were two minutes during which a buzzer was sounded. If the rat pressed the lever during the last 30 seconds of this two-minute period, it received an electric shock and the buzzer ceased. Responding was greatly suppressed during the buzzer periods, and the animals only rarely received a shock. When they did, it had little effect on their subsequent behavior. Following 0.5 mg./kg. dose of amphetamine, the rate of responding increased when the buzzer was not sounding, but suppression during the buzzer period continued. When, however, the animal did receive a shock while under the influence of amphetamine, the behavior was disrupted for several minutes. This is interpreted by Teitelbaum & Derks as evidence for an increased effect of shock in the amphetamine-treated animal. As the authors point out, it is possible that amphetamine reduced the motivational potency of food reinforcement and that, despite the generally increased

rate of responding, the behavior was therefore more fragile and readily disrupted by a shock. The fact that the rate of responding for food was generally higher following amphetamine does not eliminate this possibility, since high rates have been seen at a time when food consumption was decreased (34, 86, 146). On the basis of inspection of their animals, Teitelbaum & Derks felt there was a strong resemblance between rates given a small dose of amphetamine and then subjected to electric shock and animals given much larger doses of amphetamine, as though the shock shifted the dose-effect curve of amphetamine. Indeed, electric shock has been shown to enhance the lethality of amphetamines in rats (145), but so have a variety of other environmental factors, in mice at any rate, including some that would not be expected to function as aversive stimuli. Finally, it has been reported that after reserpine relatively normal avoidance behavior by cats may be severely disrupted when the animal receives a shock (68). It must be concluded that a specific enhancement of the effects of aversive stimulation by amphetamine is an interesting possibility, but it has not been established.

The effect of amphetamine on the performance of rats working for water on a DRL schedule has been studied by Sidman (117). A DRL schedule specifies that a response will be reinforced only when it occurs after the elapse of a minimum interval of time since a previous response. The dependent variable was the distribution of interresponse times. The peak of the distribution was markedly shifted to the left by amphetamine, i.e., interresponse times tended to be greatly shortened. In a subsequent publication (119) the results with amphetamine are said to indicate that the effect is not a "simple excitatory one," although it is not clear what this implies. Alcohol, on the other hand, reduced the height of the peak of the interresponse time distribution without changing its position. It was concluded that alcohol had relatively little effect on timing behavior. Sufficient dosage of pentobarbital led to an almost complete flattening of the distribution of interresponse times except for a large proportion of very short ones. This is interpreted as abolition of all evidence of timing behavior (118). It does not seem to the reviewers that the description of these interesting findings in terms of timing behavior is helpful.

An effect of amphetamine on discriminative behavior has been suggested (33, 118). There is no doubt that under some circumstances, the quantitative differences in behavior in the presence of two stimuli were lessened by amphetamine, and so, in this sense, amphetamine modified discriminative behavior. It is not clear at present whether this is a useful component to include in a parsimonious account of the behavioral effects of the amphetamines. A related effect appears to have been noted following ephedrine in studies on conditioned salivation (55).

This is a convenient place to describe briefly some of the work done with methylphenidate and pipradrol. The behavioral effects of both these drugs seem similar to those of the amphetamines. Methylphenidate produced

an increase in SMA of normal monkeys in a photo-cell device but only over a narrow dosage range; 1 mg./kg. produced a slight increase but 2.5 mg./kg. produced a large decrease, particularly in "pacing" as opposed to "leaping" (29). Even 1 mg./kg. produced a large decrease in activity in already hyperactive monkeys, a finding further emphasizing the dependence of this type of effect on the on-going behavior. The effects of reserpine were partially antagonized by methylphenidate in this situation (29). Total activity in rats, assessed by inspection, has been fractionated using a previously worked out scoring system, and methylphenidate has been shown to have differential effects on different components (7). The observer pressed one of six different keys depending on the category of behavior currently indulged in by the animals, thus tabulating the frequency of occurrence of the various behaviors. Methylphenidate decreased unconditioned, but increased conditioned salivation in dogs (41). Finally, the drug has been reported to facilitate reconditioning in human subjects of a galvanic skin response to a one-second visual stimulus that was usually, but not invariably, followed after 0.5 seconds by two seconds of mild shock (116).

Pipradrol has been shown to increase SMA of mice as measured in a photo-cell device (19). It has also been reported to speed the development of lever-pressing avoidance behavior in rats, without affecting escape behavior under these circumstances (128); pipradrol was studied only at a single dose level, but was compared with other drugs in these experiments. A slight decrease in running times of rats in a maze following administration of pipradrol has been reported (121). In man, it has been shown that subjects can discriminate that they have received pipradrol in doses as low as 2 mg. and that, in one subject at least, the output of verbal behavior in words per minute increased after pipradrol (52). The "thematic content" of the speech was changed concurrently.

In spite of the fact that ingestion of 10 mg. or even 5 mg. of one of the amphetamines usually produces readily discriminable "subjective" changes in human subjects—frequently in the direction of "euphoria" (4)—it has not proved easy to demonstrate substantial objective behavioral effects in unfatigued normal subjects. For example, no effect was found in performance on a motor co-ordination type of task made more difficult by being performed with mirror vision (Pursuit Confusion Test) nor on the accuracy of the predictions of the subjects as to how well they were going to perform (58). Similarly, it was without detectable effect on simple or choice reaction times and on the progressive reduction in reaction time that occurred as an arbitrary pattern of sequence of responses was acquired (75). On the other hand, consistent effects could be obtained in some of these situations in subjects who had suffered a decrement in performance as a result of fatigue resulting from prolonged wakefulness (77), and amphetamines have been shown to be able to reverse a decrement in performance that had developed during a session (103).

Nevertheless, effects can be demonstrated in unfatigued subjects. The

distribution of interresponse times of subjects working on a DRL schedule was moved significantly toward shorter interresponse times by 5 mg. of dextro-amphetamine (39). This effect is qualitatively similar to that described in rats (117), though much smaller. That the effect was smaller is perhaps not surprising since the human subjects received about 0.06 mg./kg. by mouth, while the rats received 2 mg./kg. of dextro-amphetamine intraperitoneally. Again, 10 mg. of dextro-amphetamine given four hours before the test session have been reported to increase the total time that subjects were able to keep a stylus in contact with a metal disk rotating on a turntable (44). Finally, a finding of extremely great practical importance is that of Smith & Beecher (122) who have shown that amphetamine can improve the performance of trained athletes (swimmers) already working at peak performance. Although the effects were small on a percentage basis—comparable to those seen on DRL schedule performance —they were large compared to the margins decisive in competitive sports.

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PHARMACOLOGICALLY ACTIVE SUBSTANCES OF MAMMALIAN ORIGIN[1,2]

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The limited space available for dealing with a subject of such extent and interest as this makes it very difficult to give an account of it, even a concise and approximate one. The sole purpose of this review article, then, is to stimulate the reader to look elsewhere in order to widen his knowledge of this up-to-date and fascinating field of research.

The term "pharmacologically active substances" is necessarily ambiguous, and, consequently, the choice of substances to be included under that denomination will be largely arbitrary.

We shall confine ourselves to dealing essentially with those compounds which are active on smooth muscles and blood vessels and make up the group called "local hormones" or "tissue hormones." They are compounds which carry out their predominant actions in the immediate neighborhood of their site of production. We shall not consider the true hormones (e.g., the polypeptides of the neurophypophysis) nor the neurohumoral mediators or nervous transmitters. So the catecholamines will be discussed briefly, not as neurohormones, but as possible local hormones. Similarly, only active compounds present under normal conditions will be considered, leaving aside those liberated solely under pathological conditions.

The three groups of active compounds to be taken into consideration in succession are (*a*) amines, (*b*) polypeptides, and (*c*) lipid-soluble organic acids.

AMINES

Rapid improvement of purification and detection methods of amines in biological materials and, perhaps even more, routine use of monoamineoxidase (MAO) and diamineoxidase inhibitors have led in the last few years to a conspicuous increase in the number of known biogenic amines. It may be predicted that the list of these compounds is destined to lengthen in the near future.

In this paper we will include among the biogenic amines the classic compounds, which have powerful actions on smooth muscles and blood vessels, and their derivatives still bearing the amino group in the molecule. Many

[1] The survey of the literature pertaining to this review was concluded in June, 1960.

[2] Abbreviations used in this chapter include: CNS (central nervous system); 5-HT (5-hydroxytryptamine); MAO (monoamineoxidase); PPS (pain-producing substance).

of these derivatives have been considered inactive metabolites of the authentic amines, but this opinion should be dropped because it is largely arbitrary. In fact, there is no valid reason for excluding the possibility that in certain sites or on effector structures other than smooth muscle, methylated, acetylated, or conjugated derivatives may be as active as, or even more active than their supposed precursors. Melatonin (139, 140) and psilocybin (206) are striking examples in favor of this assumption.

As already stated, this review is not intended to discuss in detail the amines involved in the transmission of nerve impulses. However, evidence is accumulating which suggests the possibility that these amines too (to be more precise, above all, the catecholamines) may also act as local hormones producing their effects independent of any direct nervous activity.

Biogenic amines are small, very agile molecules, which can be rapidly synthetized and rapidly destroyed. They have all the requirements for a quick, brief action, which may, of course, also represent the initiation of long-lasting, delayed chain reactions.

Phenylalkyl- and Phenylalkanolamines

Phenylethylamine.—In normal urine this is present in amounts less than 20 μg./day, but after MAO blockade and feeding extra phenylalanine (the precursor amino acid), this quantity increases up to 60 μg./day. Urine of phenylketonuric patients does not differ from urine of normal subjects in its phenylethylamine content, but treatment with MAO inhibitors produces an enormous increase of the amine content, up to 3000 μg./day, and more (117). Exogenous phenylethylamine possesses only moderate peripheral effects, but it is possible that these are considerably reinforced after MAO blockade and that, following administration of the precursor amino acid plus MAO inhibitors, central effects also make their appearance.

Tyramines.—All three tyramines, ortho, meta, and para, have been shown, quite recently, to be normal constituents of mammalian urine (117), and it is quite certain that in the near future they will be identified in mammalian tissues as well. This implies that the mammalian organism will also contain all three precursor tyrosines, i.e., that phenylalanine may be hydroxylated in ortho, meta, and para position (78). The enzyme capable of decarboxylating ortho- and meta-tyrosine was detected in the mammalian organism long before the detection of the tyrosines themselves (25).

The detection in mammals of tyrosines and tyramines, other than para isomers, was preceded by their demonstration in lower vertebrates and invertebrates, leptodactyline [(*m*-hydroxyphenylethyl)-trimethylammonium] in *Leptodactylus* skin (68), and *o*-tyrosine in acid hydrolysates of the larval cuticles of the insect, *Calliphora vomitoria* (56).

It may be of interest to remember that tyramine or a tyramine-like substance is a normal constituent of human saliva (175) and that N-acetyl-*p*-tyramine has been isolated from the silkworm (36). It is quite possible that N-acetyl-tyramines are also found in mammalian organisms, since they

are capable of N-acetylating catecholamines and 5-hydroxytryptamine.

Octopamine.—This amine, *p*-hydroxyphenylethanolamine, was first detected in extracts of the posterior salivary glands of octopoda (64). Now it has apparently been found in extracts of ganglia of octopoda (46) and, what is more important, in urine and several tissues of mammals treated with MAO inhibitors. In extracts of some rabbit tissues, octopamine became, after MAO blockade, the most prominent phenolic amine (119).

At present it cannot be decided whether octopamine originates from hydroxylation of *p*-tyramine or from decarboxylation of *p*-hydroxyphenylserine, an amino acid hitherto unknown in nature. Mammalian and invertebrate organisms contain enzyme systems capable of catalyzing both reactions.

Since both meta- and ortho-tyramine occur in invertebrates and mammals, it may be that even their hydroxylation derivatives, *m*-octopamine and *o*-octopamine, will be detected in the future in biological materials.

The above list of phenylalkyl- and phenylalkanolamines occurring in the mammalian organism should be considered provisional. At the Institute of Pharmacology, University of Parma, another hydroxyphenylalkylamine and another phenolic amino acid have been found on chromatograms of tissue extracts from lower vertebrates and invertebrates. Their identification is in progress.

Tyramines and octopamine do not produce particularly remarkable peripheral pharmacological effects, as they are 100 times less potent than epinephrine or norepinephrine. This statement, however, is of negligible importance because it is conceivable that on particular effector structures or enzyme systems they may be as active as, or even more active than catecholamines. Mitoma and co-workers (153), for example, found that in experimental animals, presumably after having been decarboxylated in brain tissue to the corresponding amines, intravenous ortho- or meta-tyrosine produced marked signs of central excitation and sympathetic stimulation resembling those caused by *d*-amphetamine.

Catecholamines.—It now seems highly probable that catecholamines may display in the mammalian organism functions other than those of nervous transmitters. In fact, it is possible that catecholamines are released in tissues independently of any direct nervous activity and that they act to produce, at the site of their liberation, discrete and localized responses, exactly as do authentic local hormones.

In favor of this opinion: (*a*) Nordenstam and co-workers (2, 156) have demonstrated in the connective tissue of the human skin the existence of small, eel-like chromaffin cells which are believed to store catecholamines. It is not known whether biosynthesis and release of catecholamines by these cells is controlled by the autonomic nervous system. If not, the amines would act as local hormones, possibly in the regulation of the caliber of blood vessels (35, 161b). It is of interest, in this regard, that calf skin, the vessels of which are subjected to greater hydrostatic pressure, contains two

to four times more chromaffin cells than abdominal skin.

(*b*) Falck and co-workers (23, 77) found particular chromaffin cells which were clearly distinguishable by histochemical methods from all other mammalian chromaffin cells in liver, lung, and gastrointestinal tract of cows, sheep, and goats. Since the distribution and number of these cells closely followed the dopamine content in various tissues, the Scandinavian investigators suggest that the chromaffin cells are storage structures for dopamine. The function of the huge accumulation of dopamine in ruminant tissues (up to 10 to 25 μg./gm.) is unknown, since it is unknown whether the dopamine is formed locally or is taken up from the blood (177). However, the fact that only ruminants possess dopamine-storing cells is in favor of the idea that the dopamine in the cells is a local hormone rather than a nervous transmitter.

Epinephrine and norepinephrine have been considered for years as the only active catecholamines in the mammalian organism. However, dopamine is now being considered by an increasing number of investigators not only as a precursor amine, but as a compound having its own definite physiological functions (39, 178); some of the so-called metabolites of epinephrine and norepinephrine, in their turn, are reconsidered in view of the possibility that they are not simple inactivation products.

Axelrod (9) showed that a major metabolic pathway for epinephrine and its congeners is O-methylation at the 3 position, and Evarts and coworkers (72) claimed that this O-methylation is an effective means of inactivating catecholamines. This conclusion may be exact in regard to the direct circulatory effects of catecholamines, but it seems largely arbitrary from a general point of view. Bacq (12) and Charlier (45), for example, maintain that metanephrine and normetanephrine act, through inhibition of methyltransferase, as physiological regulators of tissue sensitivity towards adrenergic mediators.

Other metabolites of catecholamines have been detected in mammalian urine; 2,4,5,-trihydroxyphenylethylamine, a metabolite of dopamine (181), and *N*-acetylnormetanephrine, a metabolite of norepinephine (186), and certainly others will be found by the routine use of MAO inhibitors and of inhibitors of the catechol O-methyl transferase. It should be added that Senoh and co-workers (182) succeeded in demonstrating that catechol O-methyl transferase is capable of catalyzing not only the *m*-O-methylation, but also the *p*-O-methylation of phenolic compounds. O-methylation of catecholamines at the 4 position (55b), instead of at the 3 position, would lead to a new series of compounds of the greatest interest.

Histamine

In spite of numerous papers and continuous interest in histamine, our present knowledge of the role of this amine in normal and pathological physiology is still largely obscure. However, it may be that the basis for a more rapid progress in histamine investigation has been laid by Kahlson

and co-workers (123) who have approached in a more dynamic way the problem of the physiological significance of the substance. Everyone will now agree with Kahlson that for the solution of the above fundamental problems, studies on the rate of histamine formation and metabolism and routine employment of drugs or diets capable of interfering in the biosynthesis, storage, and breakdown of histamine are more promising than static measurements of the histamine content of tissues. This statement, of course, can be applied generally to all other biogenic amines and, probably, to all the other active substances dealt with in this paper.

Biosynthesis, storage, and metabolism.—All the histamine appearing in tissues and urine of rats kept on a histamine-free diet is of endogenous origin. Intestinal bacteria do not contribute to its formation (95). The puzzling observation that urinary histamine is mainly in conjugated form in male rats and in free form in female rats has not received as yet any satisfactory explanation (155).

Histamine is formed in the organism by decarboxylation of histidine. This enzymic reaction occurs in a number of parenchymatous tissues, including brain (214), but it is of different intensity in different tissues and different animal species. Enormous differences could also be demonstrated between prenatal and postnatal life. In the liver of rat fetuses, for example, the histidine decarboxylase activity was 3000 to 5000 times more intense than in the liver of adult rats (124).

Histidine decarboxylase is, similarly to other decarboxylases, a pyridoxal-dependent enzyme. The consequence is that a pyridoxine-deficient diet may consistently reduce biosynthesis of histamine. The same result, of course, could be reached by the use of histidine decarboxylase inhibitors. However, pyridoxine-deficiency would necessarily impair decarboxylation of a number of other amino acids, and available histidine-decarboxylase inhibitors (e.g., semicarbazide) are unsatisfactory both in regard to intensity and specificity of effect. This makes diets and inhibitors very useful in studying the rate of histamine formation, but much less so in investigating the physiological consequences of lack in histamine biosynthesis.

Once formed, histamine may be immediately metabolized or stored. Storage, which necessarily implies a more or less strong bond between histamine and cellular constituents, may be independent of the rate of histamine formation and seems to be more conspicuous and fast in adult than in fetal life (123). As with other amines, histamine may be stored in sites different from those of its biosynthesis (e.g., blood platelets).

Histamine storage is evidently intended to meet hypernormal local or systemic requirements for the amine, under particular physiological or pathological conditions. Impairment of storage capacity could reasonably be expected to bring into light some aspects of histamine deficiency and be of usefulness in prophylaxis of pathological manifestations, resulting from abrupt release of the amine from its body depots (123). Cortisone, for example, seems to hinder storage of histamine (174).

We already know several products capable of liberating histamine from its binding sites (compound 48/80, reserpine, polymyxin B, 5-hydroxytryptophan, etc.). Unfortunately, their histamine-releasing activity varies from one animal species to another, and in the same species from one tissue to another. Moreover, none of them is a specific liberator, as release of other amines, heparin, and, presumably, other active products regularly accompanies liberation of histamine. Useful as they may prove in the elucidation of biochemical problems, this is the fundamental reason for the poor contribution of histamine-releasers to our knowledge of histamine function.

Free histamine is attacked by various enzyme systems. Although diamineoxidase seems to be the enzyme which acts most intensely and promptly *in vitro*, it is questionable whether it is the enzyme mainly responsible for the inactivation of histamine in the living organism under normal conditions. It may be that MAO plays a major role.

We now possess a growing number of drugs which inhibit diamineoxidase (aminoguanidine), MAO, or both [iproniazid and related compounds containing a hydrazine moiety (48, 90)], and it has already been shown that under the influence of these drugs histamine may accumulate in concentrations larger than normal in tissues, including nervous tissue (21), and a larger portion of the formed histamine may be excreted in the urine (128). MAO and diamineoxidase inhibitors will certainly contribute to our knowledge of histamine metabolism, but, again, it is doubtful whether they will contribute to a better understanding of the physiological significance of the amine. The reason for this doubt lies in the lack of specificity of the inhibitors and in the increasing number of biogenic amines found to be substrates for MAO and diamineoxidase.

Physiological functions of the histamine occurring outside the mast cells.—(*a*) Histamine in pregnancy: In pregnancy there is a more or less evident rise in the urinary excretion of histamine, which starts some time before term and falls just before or immediately after parturition. Although this increase in histamine production and excretion during pregnancy has been well known for a long time, it has been studied with accuracy only in the last few years by Kahlson and co-workers (120, 121). These investigators demonstrated that in rats the excess urinary histamine found in late pregnancy is of fetal origin, the fetal liver being the organ mainly responsible for the increased biosynthesis of the amine. Fetal histamine formation is considerable in man also (124).

Maternal urinary excretion of histamine falls abruptly at the time of parturition, and this fall is accompanied in the organism of the newborn rat by a rapid regression of the histidine-decarboxylase activity from an unparalleled height to the low level found in adult animals. This evidently means that excess histamine produced by the fetuses during pregnancy may have something to do with parturition or with fetal development.

In the rat in which the uterine smooth muscle is inhibited by histamine, it may be reasonably presumed that this substance might contribute to the in-

hibition of uterine contraction before term. In other species, in which histamine does not inhibit uterine motility, the function of fetal histamine ought, of course, to be different. Kahlson and co-workers (120) are of the opinion that histamine could interfere, in a way unknown as yet, in fetal development. In fact, during pregnancy, inhibition of histidine-decarboxylase brought about by administration of semicarbazide to rats fed a pyridoxine-deficient diet produced arrest of growth and death of fetuses.

(*b*) Histamine and growth processes: Kahlson & Rosengren (122) found that in the rat liver the rate of histamine formation due to a faster decarboxylation of histidine was considerably increased during the phase of regeneration following partial hepatectomy. When the regenerative growth was complete, histamine biosynthesis subsided. Furthermore, Kahlson (123) observed that the tensile strength of healing skin incisions in the rat was increased when the formation of histamine was accelerated and decreased when histamine formation was inhibited. From the above experiments, the investigators conclude that there is a strict connection between histamine formation and constructive metabolism in regenerating liver and healing wounds.

It is not necessary to emphasize the importance of the observations of the Kahlson group. Obviously, as stated by Kahlson himself, the final proof of the active participation of histamine in growth processes will have to await the findings of specific, nontoxic inhibitors of histidine-decarboxylase or of drugs capable of specifically inhibiting the coupling of histamine to its binding sites.

(*c*) Histamine as a chemical mediator in the central and peripheral nervous system: Histamine is found in very large amounts in the hypophysis (especially in the posterior lobe and in the stalk) and in the pineal gland (55b); in considerable amounts in the hypothalamus, corpus mammillare, supraoptic region, and area postrema; and in very small amounts in other parts of the brain (1, 53, 103). No histamine is present in dog cerebrospinal fluid (1). Whereas histamine extractable from the hypophysis may be, in part, of mastcell origin, this is not the case for brain histamine (1, 103). The hypothalamus is capable of forming histamine at a much faster rate than other parts of the brain (214).

Considerable quantities of histamine were also detected in optic nerves, dorsal roots (208), and postganglionic sympathetic fibers of mammals [up to 100 μg./gm. (75)] as well as in ganglia of octopods [2 to 4 μg/gm. (21)].

Although, in vertebrate brain, histamine is said to be insensible to reserpine (205), this is by no means true for the optic ganglia of octopoda. Histamine behaved in these ganglia exactly as did catecholamines and 5-hydroxytryptamine. In fact, reserpine produced a 90 per cent depletion of the histamine content, and β-phenylisopropylhydrazine, a powerful promptly acting MAO inhibitor, a 100 per cent increase of the amine level. A 70 to 100 per cent rise of the histamine concentration was observed within 30

min. after administration of β-phenylisopropylhydrazine. This means that the half-life of histamine in optic ganglia of octopoda is of 15 to 20 min., a figure of the same magnitude as that found in invertebrate ganglia and mammalian brain tissue for 5-HT (21, 200).

From a physiological point of view, it is remarkable that low doses of histamine were found to stimulate the anterior pituitary gland to secrete ACTH (83), to enhance the electrical activity of the cerebellum (53), to stimulate the hypothalamus and the superior cervical ganglion (197), and to inhibit, as intensely as 5-hydroxytryptamine, transcallosally evoked potentials in the optic cortex (91).

Histamine is now being reconsidered and re-evaluated as a possible chemical mediator of the cerebral control of the pituitary gland as well as a possible mediator in the transmission of nervous impulses within and outside the central nervous systems (CNS). Crossland (53) suggests that even the sedating action of antihistamine drugs may be related to the central effects of histamine.

Histamine in the CNS has been ignored for a long time by several investigators as a second-class amine. But this amine, however annoying the fact may be, has the same citizenship rights in the CNS as catecholamines and 5-HT, whose function in the CNS is approximately as obscure as that of histamine.

Shore and co-workers (182b) have recently proposed and employed a fluorometric method for the assay of histamine in tissues. Having found with their method that brain tissue contained very low levels of histamine (why are levels of 0.2 to 0.4 μg./gm. considered very low?) and that gross dissection of brain showed no marked localization of brain histamine, the above investigators conclude that the uniform distribution of the amine suggests that it may be associated with nonnervous, perhaps vascular, tissue. The findings of Shore and co-workers are at complete variance, in almost every respect, with those of investigators who have used biological and colorimetric assay procedures for histamine. It is evident that either fluorometric assay or bioassay are incorrect. In the writer's opinion, biological activity is something more than fluorescence, especially when this activity is determined with the critical accuracy employed by Adam (1).

Physiological functions of mast cell histamine.—Histamine has for a long time been involved in the pathogenesis of the generalized and local anaphylactic reaction, as well as in the pathogenesis of the so-called anaphylactoid reactions produced by the systemic or local application of ovoalbumin, dextran, and other high-molecular weight substances. In addition, the amine has often been considered as one of the factors responsible for the early vascular reactions to injury produced by physical and chemical agents.

The histamine implicated in these reactions is generally considered to be produced in, and released from, the mast cells. This opinion has developed since Riley (164) and other investigators (28) demonstrated that

in a number of tissues of adult mammals there was a close relationship between the number of mast cells and histamine content, the histamine concentration in different tissues of the guinea pig being 0.025 to 0.034 μg. per mast cell.

However, whereas it remains definitely established that in the guinea pig histamine has a prominent role in the acute phenomena of both systemic anaphylactic reaction and Schultz-Dale reaction, results obtained in other animal species were considerably more complicated or largely divergent, and other known and unknown substances have put forth their candidature as mediators in anaphylaxis and, still more, in processes reactive to local injury.

5-Hydroxytryptamine, for example, has been claimed to perform an important role in anaphylaxis in mice (79, 196) and, possibly, in rabbits (204), but neither histamine nor 5-hydroxytryptamine seems to play a decisive part in anaphylaxis in the rat (30).

In regard to early vascular reactions to injury, the intervention or, better, the importance of histamine is still more questionable. Spector & Willoughby (188, 189), for example, think that in the rat 5-hydroxytryptamine may be of greater importance, or as important as histamine as a mediator of increased capillary permeability in the earliest phases of acute inflammation produced by thermal injury applied on the skin or by turpentine injected into the pleura, and that neither amine interferes in the main sequence of inflammatory events. Similarly, Wilhelm (215) suggests that histamine appears to mediate, following mild thermal injury, only an immediate permeability response which is minor in the guinea pig and minimal in the rat, and that the histamine permeability effect has no necessary function as an initiation of the major delayed inflammatory responses. Finally, Smith & Miles (187) claim that both histamine and 5-hydroxytryptamine play an inessential role in early inflammation of the rat peritoneal cavity produced by bacteria and bacterial toxins.

Anaphylaxis and inflammation are complicated phenomena which cannot be explained only in terms of histamine, 5-hydroxytryptamine, or other amines. That these compounds may intervene in the above processes is possible, but their relative importance and their relationship to other shock-producing or inflammatory agents of lipidic (slow-reacting substance A) or polypeptide and protein nature [bradykinin (28c), anaphylatoxin (97), leucotaxine and exudin (151), globulins affecting capiliary permeability (152)] remains to be established.

It has been recently shown that an imidazole N-methyltransferase present in mouse liver is capable of N-methylating histamine (32), and at the same time N,N-dimethylhistamine has been detected by Bertaccini (unpublished observations) in extracts of posterior salivary glands of an octopod, *Eledone moschata*. Thus, N-methylhistamines also ought to be listed among the naturally occurring amines of possible biological interest.

5-Hydroxytryptamine (5-HT) and Related Indolealkylamines

5-Hydroxytryptamine (5-HT, enteramine, serotonin) has been the object during the last few years of intense and extensive research reported in an enormous number of publications. It is impossible to give here even a concise account of these studies [see review articles by Page (158), Lewis (143, 143b), Levy (142), Erspamer (65, 71), etc.], and as a consequence, attention will be focussed only on a limited number of topics of the 5-HT investigation, above all on the problem of the biological significance of 5-HT in the mammalian organism.

Occurrence and distribution of the enterochromaffin cell system and 5-HT in mammals.—The enterochromaffin cell system, and hence 5-HT, is present in the gastrointestinal mucosa of all the examined vertebrate species, including all classes of fishes, as well as in the gastrointestinal tract of the ascidians and of *Amphyoxus lanceolatus* (202b). The recent finding that even the intestinal mucosa of *Teleostei* may contain, at least in restricted areas, a limited number of enterochromaffin cells [Erspamer (unpublished observations)] has filled a hitherto incomprehensible gap and points to a general biological significance of the 5-HT produced by the enterochromaffin cells.

In addition to the gastrointestinal mucosa, 5-HT is also present in considerable amounts in blood platelets and in organs which capture blood platelets (spleen, occasionally lung) as well as in the central nervous system. The substance has, in addition, been found in mast cells and, consequently, in all tissues of rats and mice containing mast cells (49, 66).

Two other indolealkylamines have been shown in the last few years to be normal constituents of the mammalian organism: tryptamine, present in very low concentrations in urine and in several parenchymatous tissues, including brain (107), and melatonin (5-methoxy-N-acetyltryptamine), present, again in very minute amounts, in the pineal gland and in peripheral nerves of mammals (139, 140, 141b).

Biosynthesis and metabolism of indolealkylamines.—It is generally accepted that the enterochromaffin cells of the gastrointestinal mucosa are the main site of production and storage of 5-HT in mammals (55, 65, 66). These cells contain both the enzyme systems which catalyze the two-step passage leading from dietary L-tryptophan to 5-HT. Evidence for this assumption is given, on one hand, by the enormous increase in 5-HT, and eventually 5-hydroxytrytophan production in cases of malignant argentaffinoma (195) (i.e., in cases of neoplastic proliferation of the 5-HT-secreting cells) and, on the other hand, by the conspicuous decrease in 5-HT metabolism following removal of large segments of human (19, 105, 106), dog (69, 169), and rat intestine (20), i.e., following reduction of the 5-HT-secreting tissue. Blood and spleen 5-HT originates, at least to its greatest extent, from the gastrointestinal mucosa.

As for mast cell 5-HT, it now seems certain that the substance may be synthetized entirely within the mast cells themselves. This has been shown

by Schindler and co-workers (176), who found that neoplastic mast cells obtained from a mouse mastocytoma maintained, even after many generations in culture, their high intracellular level of 5-HT. However, it appears once again necessary to emphasize the fact that only in rats and mice do normal or pathological mast cells contain 5-HT. No detectable amounts of 5-HT could be found in mast cells from other mammals (159). The consequence is that, from a general point of view, 5-HT in mast cells of mice and rats is little more than a biochemical rarity, exactly as is the occurrence of 5-HT in the hypobranchial gland of some prosobranchiate molluscs or in the body of some coelenterates (65, 149).

The last extremely important and constant example of 5-HT in the mammalian organism is brain 5-HT. The problem of the origin of cerebral 5-HT is not completely solved. In fact, even if it is certain that brain tissue can decarboxylate 5-hydroxytrytophan, there is no direct evidence that this tissue is also capable of carrying out the oxidation of L-tryptophan into 5-hydroxytrytophan. Indirect evidence, however, seems to strongly support the view that even brain 5-HT may originate locally from L-tryptophan. We refer to the occurrence of considerable amounts of 5-HT in ganglia and peripheral nerves of molluscs and crustaceans apparently lacking the amine in all other tissues (66) and to the presence of 5-HT in the brain of rats deprived operatively of the whole gastrointestinal tract (20).

There is no doubt that the limiting step in the 5-HT biosynthesis is the hypdroxylation of tryptophan to 5-hydroxytrytophan, catalyzed by a tryptophan hydroxylase which apparently has now been traced to the mammalian gastrointestinal mucosa. Decarboxylation of 5-hydroxytrytophan is carried out in a number of tissues by a widespread, poorly specific decarboxylase, which is indistinguishable from dopadecarboxylase (22, 169b, 209), and from the enzyme decarboxylating 4-hydroxytryptophan (70).

Tissue tryptamine is formed by decarboxylation of L-tryptophan, possibly catalyzed by the same enzyme which decarboxylates 5-hydroxytrytophan (201); melatonin derives from O-methylation (provoked by an indole O-methyltransferase) of N-acetyl-5-HT, which may be considered a normal metabolite of 5-HT (150). It has been claimed that the indole O-methyl transferase is a specific enzyme, strictly localized in the pineal gland (10).

Once formed, 5-HT may be stored in the cells in which it has been synthetized or may be released in a free form into the interstitial liquids or into the plasma. The free 5-HT, which is the active form of the amine, is promptly subjected to attack by several enzyme systems acting both on the lateral chain and on the indole ring. Monoamineoxidase causes the oxidative deamination of 5-HT with formation of 5-hydroxyindoleacetic acid; ceruloplasmin attacks the phenolic hydroxy group with possible formation of a *p*-quinone imine derivative (163); other enzyme systems produce acetylation of the amino group in the lateral chain, or catalyze O-conjugation with glucuronic acid (207) or, to a lesser extent, with sulphuric acid (41, 150). N-methylated derivatives of 5-HT, as found in amphibian skin, are not known in the mammalian organism.

Following administration of radioactive 5-HT to rats, approximately 40 to 90 per cent of the radioactivity was found in urine in the form of seven radioactive compounds giving a positive *p*-dimethylaminobenzaldehyde reaction (127, 150).

Monoamineoxidase seems to be the enzyme system mainly responsible for the inactivation of tryptamine, but, besides oxidative deamination, hydroxylation of the indole ring at the 6 position should also be considered as an alternate metabolic pathway for the amine (116). The principal pathway of metabolism of melatonin is 6-hydroxylation, followed by O-conjugation of the N-acetyl-5-methoxy-6-hydroxytryptamine with glucuronic and with sulphuric acids (131).

An enormous contribution has been afforded in the study of the biosynthesis and metabolism of indolealkylamines by the routine use of MAO inhibitors. They have been of decisive importance, for example, in the detection in tissues or urines of tryptamine and several 5-HT metabolites, as well as in the determination of the turnover rate of 5-HT and tryptamine in the central nervous system. It is to be expected that dopadecarboxylase inhibitors will also help to solve many problems connected with the biosynthesis of tryptamine and 5-HT.

The limiting step in the biosynthesis of 5-HT is, as already stated, the hydroxylation of L-tryptophan at the 5 position. The tremendous interest afforded by a better knowledge of the apparently specific enzyme system involved in the reaction is obvious, as it is obvious that the eventual discovery of specific inhibitors of the L-tryptophan hydroxylase would probably offer the key to the understanding of the function of 5-HT within and outside the cental nervous system.

Physiological significance of 5-HT.—(*a*) 5-HT of enterochromaffin origin: Under normal conditions, a quantity of 5-HT corresponding to that contained in the gastrointestinal mucosa is synthesized by the enterochromaffin cells in 8 to 20 hr. (69, 200). From the enterochromaffin cells 5-HT is released into the interstitial liquids and then into the plasma where it is largely absorbed by the platelets. It it highly probable that very minute amounts of 5-HT circulate free in the plasma and that platelets can liberate an aliquot of their 5-HT without necessarily being disrupted or altered.

It is evident that 5-HT may display its biological actions either immediately after its release while still in the interstitial liquid surrounding the enterochromaffin cells or after penetration into the circulatory stream. In the first case, 5-HT should be regarded as a local hormone, in the second as a true hormone.

Smith and co-wokers (185) suggest the possibility that 5-HT "has no normal role as a circulating hormone," and this opinion is largely shared by Lembeck (137), who similarly believes that 5-HT displays mainly local actions, because the other pharmacological effects of the substance become apparent only after injections of exogenous 5-HT or following hyperproduction of 5-HT by malignant argentaffinomas.

The hypothesis of a local function of 5-HT within the gastrointestinal mucosa soon after its release from the enterochromaffin cells is at first sight very attractive, and it has been recently substantiated by important results obtained following introduction of very low doses of 5-HT and 5-hydroxytrytophan into the lumen of the isolated guinea-pig ileum. According to Bülbring and her co-workers (33, 34) and to Lembeck (137), 5-HT could stimulate intestinal motility by sensitizing the sensory receptors in the intestinal mucosa which trigger the peristaltic reflex, and, as a consequence, the formation of 5-HT by the enterochromaffin cells might be a part of the physiological mechanism required for peristalsis.

However, once again extreme caution seems to be necessary in generalizing results obtained in one animal species or in transferring experimental data achieved on isolated intestines (or even on intestinal loops *in situ*) to the gastrointestinal tract of the intact animal. In fact, if we admit that the 5-HT which enhances the peristaltic reflex has a local origin, then it would be rather difficult to give an acceptable explanation of the conspicuous differences in the distribution of the enterochromaffin cell system and of 5-HT along the gastrointestinal tract of the different vertebrate species. What can be the meaning of the enormous accumulation of enterochromaffin cells and 5-HT in the intramural portion of the pancreatic duct of the rabbit? What is the reason for the predominant accumulation of 5-HT in the stomach of mice, hedgehogs, dogs, in the small intestine of guinea pigs and chickens, and in the large intestine of rats, horses, and cows? What is the significance of the enterochromaffin cells found in the mammalian pancreatic islets and in the thymus of some birds (65, 66)?

Moreover, Smith and co-workers (185) were unable to find "any evidence for alimentary control of the release of 5-HT from argentaffinoma and from the normal argentaffin cells," and Johnsen and co-workers (118) failed to observe any relationship between meals (i.e., motor and secretory activity of the bowels) and the peak in the diurnal variation in 5-HT metabolism, as inferred from the diurnal variation in the urinary excretion of 5-hydroxyindoles.

Finally, although 5-HT introduced into the lumen of an isolated guinea-pig intestine produced a stimulation of peristalisis in very low concentrations, a very high dosage was required to produce short bursts of peristalsis when introduced into a loop of the guinea-pig intestine *in situ* (34), and no intestinal or systemic response was elicited when it was given intrajejunally to normal volunteers in doses up to 10 to 15 mg. (47). This suggests that the mucosa of the isolated intestine had undergone some alterations and that results obtained under such unphysiological conditions cannot be extended to the intestines of the intact animal.

Of course, 5-HT could act on the gastroinestinal tract also via the blood. But, frankly, it would be rather surprising if the 5-HT released from the intestines had to return to the same intestines, after having been exposed to severe enzyme attacks and to large losses, to display there its main physiological action.

At any rate, even if there is as yet no proof that physiological doses of 5-HT may influence the gastrointestinal secretory activity, there are experimental results which demonstrate that stimulation of intestinal motility may be produced by physiological amounts of 5-HT injected intravenously or intraarterially (34, 184b). These positive findings are certainly of great importance, but it is evident that they must be reproduced on *in situ* intestines of numerous animal species, including lower vertebrates, before they can be taken as convincing evidence that 5-HT has a general significance as a hormone participating in the control of intestinal motility.

Besides that just now discussed, several other theories have been advanced in an attempt to elucidate the biological significance of extracerebral 5-HT. All these theories, which see in 5-HT a factor influencing hemostasis, or consider that it controls vascular tone and, therefore, the systemic blood pressure, or, finally, regard the substance as a hormone participating in the regulation of renal function, have been critically reviewed in preceding papers (65, 67, 158). Here we wish only to call attention to a few more recent data.

The belief that 5-HT is involved in the process of blood coagulation, in the vasoconstriction subsequent to vascular injury, or, in capillary resistance has been further impaired (*a*) by the observation that capillary resistance and clotting time remained unchanged in reserpine pretreated animals (219) or humans (104), the blood of which contained as little as 2 to 5 per cent of normal 5-HT, as well as in pyridoxine-deficient chickens, the blood of which contained similarly less than 10 per cent of normal 5-HT (199); and (*b*) by the circumstance that there was no correlation whatever in human hemorrhagic diseases between tendency to spontaneous hemorrhage, on the one hand, and platelet or blood 5-HT levels and urinary excretion of 5-hydroxyindoleacetic acid, on the other (190).

The theory that 5-HT controls vascular tone is reinvigorated from time to time by new experimental and clinical data but, unfortunately, results and conclusions reached by the different research workers are often exactly opposite. In accordance with Page (158), Gordon and co-workers (92) suggest that a "biological role of serotonin lies in its negative interaction with adrenalines," and that 5-HT may decrease small vessel tone "through its capacity to antagonize the vasoconstrictor action of noradrenaline on small vessels." Woolley & Shaw (217), on the contrary, continue to maintain their original view that 5-HT is a hypertensive agent, possibly involved in human hypertensive disease, and consider as strong evidence for this assumption the fact that some of their 5-HT antimetabolites reduce or abolish not only the increase of blood pressure produced by exogenous 5-HT in dogs, but also spontaneous hypertension in human beings (216).

Actually, it is by no means possible to exclude that 5-HT may act physiologically on the vascular smooth muscle, at least in some areas, but we are at present unaware of the importance, type, and direction of this action,

and there is no evidence whatever that 5-HT may be involved directly in the pathogenesis of human hypertensive disease.

There is nothing new concerning the theory that 5-HT is a hormonal product participating in the regulation of the intrarenal circulation and the function of the kidney. In spite of the fact that the existence of an antidiuretic effect of 5-HT has been generally confirmed in all mammals studied, we must admit that so far only for rats and mice do the renal actions of 5-HT fulfill the main conditions necessary for being considered "physiological." In this respect, some experimental results of Bertaccini (unpublished observations) may be of interest. This investigator succeeded in demonstrating that oral administration of L-tryptophan produced in rats significant antidiuretic effect, probably attributable to excess production and release of 5-HT by the enterochromaffin cells.

(*b*) Mast cell 5-HT: It has already been pointed out that only mast cells of rats and mice contain detectable amounts of 5-HT. It is, of course, quite possible that mast cell 5-HT may participate in these species (together with histamine, polypeptides, proteins, and lipid-soluble organic acids) in the local regulation of caliber, permeability, and resistance of blood vessels and, hence, in the pathogenesis of the anaphylactic and anaphylactoid reaction (171, 213), and also, possibly, in the pathogenesis of the early manifestations of inflammatory reaction (188, 189). Nobody, however, succeeded in demonstrating that 5-HT can display the same local actions in other species lacking 5-HT in their mast cells. Consequently, mast cell 5-HT remains for the present a characteristic peculiar to some rodents, with no general biological significance.

(*c*) 5-HT in the central nervous system: This is the localization of 5-HT in the mammalian organism which presently raises the greatest interest. So far, more than 350 papers directly or indirectly pertaining to this subject have been published, and it is often extremely difficult to evaluate exactly the reliability and accuracy of experimental results and the consistency and solidity of interpretations and hypotheses.

Our opinion is that 5-HT must certainly possess important and, perhaps essential functions within the central nervous system. In this we agree with Gaddum (5, 85), Woolley & Shaw (218), and all other research workers who have investigated the problem of brain 5-HT. However, things appear rather complicated when trying to give a solid experimental basis to the above opinion and, still more, when attempting exactly to define and delimit the central functions of 5-HT.

The pieces of evidence which have been brought in favor of the hypothesis which sees in 5-HT a neurohumoral transmitter are as follows:

(*1*) Occurrence of 5-HT within the central nervous system is a fundamental observation. 5-HT seems to be present not only in the brain of all vertebrates examined, but also in ganglia and peripheral nerves of molluscs and crustaceans, and it is highly probable that neural 5-HT is of autochtonal origin. Another important circumstance is that, at least in mammals,

5-HT is preferentially contained in peculiar areas of the brain (brain-stem structures, rhinencephalic structures, neostriatum), some of which are part of, or are functionally connected with the autonomic system or with the reticular activating system.

Several investigators have laid emphasis on the occurrence, in the same central areas containing 5-HT, of the enzyme systems which synthetize or inactivate 5-HT. Unfortunately, the demonstration that neural tissue is capable of converting L-tryptophan to 5-hydroxytrytophan is as yet lacking. The presence in the brain of amineoxidase and of dopadecarboxylase is apparently of much less importance, because of the widespread and irregular distribution of these enzyme systems in parenchymatous tissues.

(*2*) Turnover rate of 5-HT in the central nervous system is another basic observation. With the aid of powerful and promptly acting amineoxidase inhibitors it was demonstrated that the half life of cerebral 5-HT is not longer than 10 to 30 min. (200). Similar figures were obtained for the 5-HT present in the optic ganglia of octopoda (21). This high rate of turnover fits very well the assumption that 5-HT is a neurohumor.

(*3*) Another set of experimental results strongly suggesting that 5-HT plays a role in controlling nervous processes has been afforded by the use of 5-hydroxytrytophan, the immediate precursor of 5-HT. It is certain that 5-hydroxytrytophan is capable, in contrast to the deriving amine, of penetrating into the central nervous system, and it has been demonstrated that 5-hydroxytrytophan is there decarboxylated to 5-HT. In fact, brain 5-HT may be increased several times after injection of large doses of 5-hydroxytrytophan, and a further increase occurs when MAO inhibitors are first administered.

These facts are now firmly established, but, surprisingly enough, there are still substantial discrepancies not only in the interpretation, but even in the description of the effects elicited by the huge increase in brain 5-HT obtained in this way. Some investigators observed a marked central stimulation (*27*), others an evident central depression (143b, 147). It is clear that discrepancies, while stimulating further studies, tend at present to obscure the problem of the central actions of 5-HT.

In spite of these reservations, it appears that the theory that 5-HT is involved in the control of activity of the central nervous system has a solid basis in the three groups of observations above—only in these three, however, since many other experimental results, at first sight very brilliant and unequivocal in their interpretation, have revealed their real inconsistency and ambiguity on further investigation. Examples of this are the results obtained with reserpine and the MAO inhibitors. In fact, reserpine liberates not only 5-HT and related indoleaklylamines, but also catecholamines, histamine, ATP (130), and perhaps other active products; amineoxidase inhibitors, in their turn, block the oxidative deamination of a number of biogenic amines which are substrates for MAO. In addition, it may be that

these drugs act on other enzyme systems. All this, of course, makes it very difficult to ascribe the effects of reserpine to the lack of one amine rather than to that of the other and, similarly, to attribute the actions of MAO inhibitors to an excess of one amine rather than to that of another. It may be seen that everyone interprets his results as he likes.

So far, no light can be thrown on the problem in discussion by the use of 5-HT-antagonists. Thus, it seems probable that the central effects of *N,N*-diethyl-D-lysergamide, to quote the major representative of 5-HT antagonists, have nothing to do with the anti-5-HT action of this drug (170).

5-HT has often been introduced, alone or with other drugs, into the lateral ventricles of the brain of experimental animals and humans; similarly, it has been injected intravenously or into the carotid or vertebral artery in order to study its effects on spontaneous and evoked electrical activity of different areas of the brain, on synaptic transmission in the lateral geniculate nucleus, on transcallosal transmission, on mono- and polysynaptic spinal reflexes, and on somatic and visceral behavior (71).

Unfortunately, results of these experiments were often contradictory. On the whole, inhibition and depression seemed predominant, but even facilitation and stimulation were frequently observed. It should be added (*a*) that the administered doses of 5-HT were often quite unphysiological; (*b*) that, with a few exceptions, the possibility could not be ruled out that 5-HT might act indirectly through local changes in caliber or permeability of cerebral blood vessels, or through discharges evoked from peripheral receptor organs or central chemoreceptors possibly lying outside the blood brain barrier (31); (*c*) that there is no concordance of opinion in regard to the problem of the relationship of 5-HT to the other neurohumors.

It is obvious that our limited knowledge of the physiological significance of 5-HT makes it very difficult to understand its possible role in psychiatric disorders (129). It may well be that 5-HT intervenes, alone or, much more likely, together with catecholamines and other substances, in the production of mental diseases. However, in this regard we have to consider the possibility that qualitative aberrations in the biosynthesis of 5-HT (e.g., formation of N-methylated derivatives or of derivatives bearing the phenolic hydroxy group in 4, 6, or 7 position instead of in 5) may play a more important part than hyperproduction or deficiency of 5-HT.

Physiological significance of tryptamine and melatonin.—The significance of tryptamine in the organism is completely obscure. It may be, that similarly to 5-HT, it plays a role in the central nervous system.

Melatonin is known to possess a tremendously powerful lightening action on the melanocytes of the skin of *Rana pipiens* (threshold dose 10^{-6} to 10^{-7} μg. per ml.) and to reverse the action of the melanocyte-stimulating hormone. Lerner and co-workers (140, 141) suggest that melatonin may be a neurohormone interfering with the transmission of nerve impulses and of significance in the pathogenesis of vitiligo and malignant melanomas.

POLYPEPTIDES

Angiotensin

Angiotensin (hypertensin, angiotonin) is a vasoconstrictor polypeptide produced by the action of renin, an enzyme, upon a substrate contained in the α-2 globulin fraction of the plasma.

Origin and release of renin.—Cook & Pickering (50, 51) and Nairn and co-workers (154) succeeded in demonstrating, by ingenious techniques, that in the rabbit kidney renin is located in or very close to the glomeruli, more precisely in a structure at the vascular pole of the glomerulus, possibly the juxtaglomerular apparatus of Goormaghtigh. The outer glomeruli always contained three to six times more renin than the inner glomeruli.

Nothing is known about the release of renin from the kidney under normal conditions. It has been reported that blood of normal dogs contains approximately 0.02 to 0.05 Goldblatt units of pressor substance (equalling 0.004 to 0.01 μg. angiotensin II) per liter, and blood of normal human beings 0.015 units per liter (183), but these findings could not be confirmed by other research workers (160).

Origin and fate of angiotensin.—Renin produced by the kidney cortex acts upon a substrate contained in the α-2 globulin fraction of the plasma (angiotensinogen, hypertensinogen, angiotonin precursor). Skeggs and co-workers (183, 184) succeeded in demonstrating that, unlike all other known enzymes, renin acts between two leucine residues in the middle of a very long polypeptide chain. This achievement was preceded by the demonstration that trypsin releases from the protein substrate of renin a tetradecapeptide (which soon after was also prepared by synthesis) capable of yielding the decapeptide angiotensin I on treatment with renin.

The result of the action of renin on its protein substrate from horse or ox plamsa is an inactive decapeptide called angiotensin I. This is then converted to an active octapeptide, angiotensin II, by the action of a proteolytic enzyme contained in the blood plasma having the unique property of absolutely requiring a monovalent anion for its activation (183).

In vivo angiotensin I is approximately as active as angiotensin II because its conversion to angiotensin II by the plasmatic angiotensin-converting enzyme is very rapid. Once formed, angiotensin is rapidly destroyed in blood and tissues by one or more proteolytic enzymes (angiotensinase, hypertensinase). Destruction may be avoided, and this is very important both in the bioassay and in the large scale preparation of angiotensin by acid treatment of the plasma before addition of renin, or by partial purification of the substrate or by carrying out the incubation of plasma with renin in the presence of charcoal which absorbs angiotensin (61).

Chemical constitution of the angiotensins.—Isolation, purification, and elucidation of the amino acid composition and amino acid sequence of ox and horse angiotensins were accomplished independently in 1956–57 by two groups of research workers (60, 183). The proposed structure of the

polypeptides was soon confirmed by synthesis (179, 180).

It was shown that both horse and ox angiotensins I were decapeptides differing from each other only in the amino acid residue situated in the fifth position from the N-terminal residue. This amino acid is valine for ox angiotensin (val^5-angiotensin) and isoleucine for horse angiotensin (ileu^5-angiotensin).

The amino acid sequences for the three natural angiotensins which have been isolated so far are as follows:

Ox hypertensin I: Asp. Arg. Val. Tyr. Val. His. Pro. Phe. His. Leu.

Horse hypertensin I: Asp. Arg. Val. Tyr. Ileu. His. Pro. Phe. His. Leu.

Horse hypertensin II: Asp. Arg. Val. Tyr. Ileu. His. Pro. Phe.

The plasma enzyme converting hypertensin I to hypertensin II removes the two terminal amino acids, histidine and leucine, as one dipeptide and not individually. Several synthetic analogues of hypertensin II have been prepared by Schwyzer (180).

The pharmacological actions of angiotensin have now been thoroughly re-examined using pure synthetic angiotensin preparations.

Extravascular smooth muscles.—Like various other polypeptides, synthetic angiotensin strongly stimulates the isolated intestines and uteri of several mammalian species. For the guinea pig intestine a linear dose-response relationship was observed in the range of 0.001 to 0.01 μg/ml. Sometimes the sensitivity of the muscle preparation sharply decreased after a maximum stimulus, simulating tachyphylaxis (94).

Blood pressure.—The characteristic effect of angiotensin injected intravenously is a sharp rise of the blood pressure. The shape of the pressure curve is similar to that produced by epinephrine, but shows no secondary fall below the initial level. The pressor action of angiotensin is demonstrable in all examined species, the rat being the most suitable laboratory animal for the quantitative evaluation of this activity. An appreciable rise of blood pressure may be obtained in dogs and rats with doses of angiotensin as low as 0.01 to 0.03 μg./kg., the polypeptide being about 20 times more potent than norepinephrine (94). In human beings the minimum active dose of angiotensin was found to be of the order of 0.5 μg. (183). Continuous infusion of angiotensin in rats and dogs resulted in an elevation of blood pressure during the infusion and a rapid return to the preceding level after its cessation.

Different vascular areas.—Coronary blood flow of cats was either unaffected or increased concomitantly with the pressure rise produced by doses of angiotensin between 0.01 to 1 μg./kg. (94); similarly, there was no reduction of flow in the dog pulmonary vessels (38), in the cat femoral artery (94), nor in the human forearm (160). In contrast to these vascular areas, skin vessels (37, 160), renal arteries, and, still more, mesenteric arteries were constricted by angiotensin (94). It is mainly because of its effects on the vessels in the splanchnic areas that angiotensin produces systemic hypertension.

Isolated heart.—The isolated heart of rabbits and cats responded to hypertensin with a diminution of coronary flow and a slight increase of the amplitude of beat (94).

Diuresis.—In a dosage range in which hypertensin increased blood pressure (infusion of 0.1 to 1 μg./kg./min.), the polypeptide produced a definite antidiuretic effect in hydrated animals. With low doses of angiotensin, sodium and potassium excretion were diminished, especially at the beginning of infusion; with high doses sodium excretion rose markedly during the infusion, the excretion of potassium being only slightly elevated or remaining unchanged (94).

In human beings doses of angiotensin as low as 0.01 μg./kg./min. were found to produce antidiuresis accompanied by a decrease in *p*-aminohippuric acid and insulin clearances, pointing to a vasoconstriction in the renal arterioles (26). With larger doses of angiotensin (0.1 μg./kg./min.) it was almost possible to stop urine flow. In contrast with the increased sodium excretion observed in dogs, there was in every case a marked reduction of electrolyte excretion (160). From the available data it cannot be decided whether angiotensin has an antidiuretic action independent of its vasoconstrictor effect.

Numerous angiotensin-like polypeptides have already been synthetized and studied pharmacologically in comparison with the natural products. An analysis of the relationship between their structure and their activity has been carried out by Elliott (61) and by Gross & Turrian (94). On the whole, it has been found that even rather large variations in the structure of the polypeptides have little or no influence on their specific action (94) and that amino acid sequence is not necessarily as important as size and shape of the molecule in the determination of biological activity (61). Considering more closely the naturally occurring polypeptides only, it has been demonstrated that the octapeptides are about two to four times as active as the corresponding decapeptides.

Physiological and physiopathological significance of the renin-angiotensin system.—The possible role of the renin-angiotensin pressor system under physiological conditions is largely obscure. Skeggs (183, 184) suggests that this system, by virtue of its ability to produce constriction of the arteriolar bed of the glomerulus, may have the normal function of controlling glomerular blood pressure and filtration rate. However, as Gross & Turrian (94) rightly point out "the fact that it is possible to provoke by renin the appearance in plasma of a pressor polypeptide does not yet prove that this polypeptide has any physiological significance." It has already been noted that the occurrence of angiotensin in normal plasma, even in that returning from the kidney, has been questioned (160).

The renin-angiotensin system has been considered by several investigators as the cause of the raised blood pressure in experimental renal hypertension resulting from the constriction of the main renal arteries of animals as well as the cause, or one of the more important causes, of raised blood

pressure in patients with malignant hypertension. However, the renin theory of malignant hypertension was decidedly rejected by other authoritative research workers.

Concerning experimental renal hypertension, the pieces of evidence put forward by the sustainers of the renin theory are briefly as follows: (*a*) Hypertension following the constriction of the renal arteries is caused by a chemical agent released into the blood stream, as shown by the fact that removal of the ischemic kidney produces a drop in blood pressure. (*b*) This agent ought to be renin because ischemic kidneys are loaded with renin, because there is no other pressor material known which will maintain hypertension, and because (and this seems to be the most important point) blood of renal hypertensive animals contains a conspicuously increased amount of angiotensin (0.1 to 1.6 units per liter instead of the normal 0.02 to 0.05 units). Such an amount would be quite sufficient to explain the elevation in blood pressure observed in this condition (183).

However, the most important of the above positive findings could not be confirmed in careful control experiments by Peart (160). Samples of blood were collected by catheter directly from the renal vein of rabbits with hypertension caused by renal artery clipping. At no time was it possible to show the presence of renin or angiotensin in the renal vein blood samples. Similarly, Blaquier and co-workers (24b) succeeded in demonstrating, in cross-circulation experiments in rats, that whereas immediate hypertension which follows release of clamps that had occluded one or both renal pedicles for four hours might be humorally mediated, the maintenance of the elevated blood pressure in chronic renal hypertension was not dependent on circulating pressor material.

In regard to human malignant hypertension, the claims to have demonstrated an increased amount of angiotensin in the circulating blood of hypertensive patients are again more or less balanced by negative findings. Whereas Skeggs and co-workers (125, 183) affirm that the average content of angiotensin in patients with benign essential hypertension is twice, and in patients with malignant essential hypertension 20 times that of normotensive controls, Peart and co-workers (160) were unable to detect any angiotensin in the plasma of blood collected directly from the renal vein of a wide range of patients with severe hypertension, including those with malignant hypertension and unilateral renal disease. In addition, no differences could be found between normal and hypertensive patients in the rate of formation of angiotensin following addition of renin to plasma.

Other pieces of indirect evidence unfavorable to the hypothesis that angiotensin is the agent responsible for human renal hypertension consist of the facts that although intravenous infusion of angiotensin lowers the skin flow, the hand blood flow in hypertensive patients is quite normal and that, in sharp contrast to the effect produced in normal subjects, angiotensin produces in patients with severe renal hypertension a rise in urine flow and electrolyte excretion (160).

It is evident that the above sharply contrasting experimental results make practically untenable any theory concerned with the physiological and physiopathological meaning of the renin-angiotensin system. However, negative findings, although sometimes disconcerting, are opposed by an impressive number of positive results which cannot be ignored or underevaluated. That the renin-angiotensin system is the only factor responsible for experimental and human renal hypertension seems very doubtful, but it is quite possible that it interferes with the mechanism involved in the establishment and maintenance of hypertension. It may be hoped that the present large availability of pure angiotensin will help to elucidate this extremely fascinating problem.

Kallikreins

Kallikreins are high-molecular weight, undializable, thermolable proteins which may be obtained from precursors (kallikreinogens) by the action of trypsin or other proteolytic enzymes.

The kallikreinogens contained in different tissues or fluids of the body are not identical to each other because they give origin, by incubation with trypsin, to different kallikreins. These may be distinguished from each other not only on the basis of their susceptibility to inactivators (e.g., soybean-trypsin inactivator) and to reactivating procedures, but also through their ability to release kallidin from blood plasma. Bird kallikrein, for example, cannot liberate kallidin from the serum of mammals, and guinea pig salivary kallikrein fails to release kallidin from its own plasma (82, 173, 210).

The main sources of kallikreinogens in the mammalian organism are blood plasma, pancreas, submaxillary glands, intestinal tract (especially large intestine), and, possibly, brain. The kallikreinogen of blood plasma seems to be very similar or identical to intestinal kallikreinogen, but different from pancreatic and salivary kallikreinogens. Normal urine contains only active kallikrein which derives from plasma kallikreinogen activated by the renal tubules. Following renal tubular injury in rats or nephrosis in human patients, kallikreinogen appears and active kallikrein is reduced in urine (211). The existence of different kallikreinogen and kallikrein systems is also demonstrated by the fact that various diets had different influences on the kallikrein content of different organs (80).

The concentration of kallikreinogen in plasma and intestine of dogs and rats was not altered either after pancreatectomy (210) or after total atrophy of the pancreas produced by ligation of the pancreatic duct (80). This demonstrates that the pancreas cannot be the source, or the main source, of the kallikreinogen of the plasma or of the gut, and hence of urinary kallikrein, and excludes at the same time the possibility of an endocrine secretion of pancreatic kallikreinogen (80). Similarly, the extirpation of the submaxillary glands of mice and dogs had practically no influence on the daily kallikrein output in the urine (212). On the other hand, in a series of experiments the external secretion of pancreatic kallikrein

was shown to parallel the secretion of other pancreatic digestive enzymes, more precisely that of proteolytic enzymes (80).

It appears from the above that blood kallikreinogen has its origin in other parts of the body than in the pancreas or salivary glands. Because of the strict resemblance or identity between plasma and intestinal kallikreinogens, it would seem advisable to check the intestine as possible source of the blood kallikreinogen.

When put into contact with blood, kallikrein acts both as an enzyme releasing from the pseudoglobulin fraction kallidin (a dializable, powerful smooth muscle stimulant principle) and as substrate for proteolytic blood enzymes (the so-called kallikrein-inactivators) which provoke its inactivation. It is clear that the yield in kallidin by kallikrein is much higher when the kallikrein-inactivators have been previously destroyed (82, 166).

Kallikreins alone are inactive on isolated smooth muscle preparations; they were, however, found to be extremely effective in increasing capillary permeability to a circulating dye in the guinea pig, and to possess a powerful hypotensive action when injected into the animal, especially dog (24, 82). It is a matter of controversy whether these actions are also, at least in part, attributable to the molecule of kallikrein itself or only to the kallidin derived from it (11).

The biological significance of the kallikreins is completely obscure, and it may be that it is different for the different kallikreins. Schachter (173) suggests that pancreatic and salivary kallikreins, which are in many respects analogous to other digestive exocrine secretions, may display their physiological role "inside" rather than "outside" the gastrointestinal tract. The opinion that kallikrein has some significance in shock is untenable, because kallikrein was not capable of producing in dogs the main features of the shock syndrome (205b).

Bradykinin-Like Popypeptides

Active polypeptides of low molecular weight, producing hypotension and stimulation of extravascular smooth muscles, are released by the action of different enzymes on precursors existing in blood plasma. These active compounds are the bradykinin-like polypeptides. Because there are still considerable discrepancies of opinion in regard to the unity or multiplicity of these polypeptides, it seems advisable to first list them.

Origin of the bradykinin-like polypeptides.—The polypeptide bradykinin is released from an inactive precursor, bradykininogen, present in the α_2-globulin fraction of the plasma, by incubation with trypsin or the venom of *Bothrops jararaca* (166, 167), as well as by activation of the plasminogen-plasmin system of the plasma by simple application of heat and changing the pH (165). According to Hamberg & Rocha e Silva (98) the release of bradykinin is related to the esterase activity of trypsin and of the venom of *B. jararaca* rather than to the proteolytic activity of these materials.

Kallidin is liberated from an inactive precursor occurring in plasma,

kallidinogen, by kallikrein. However, it should be stressed that not every kallikrein is capable of acting on every kallidinogen, and that it may happen that a kallikrein is inactive even on the blood kallidinogen of its own species. It seems that kallikrein, too, is not a proteolytic enzyme but rather an esterolytic enzyme of the type found in snake venom (166).

A kallidin-like substance is released from plasma or serum of some mammals by simple dilution with distilled water. Dilution probably activates serum kallikreinogen to kallikrein which then releases kallidin (172, 173).

Plasma-kinin is produced from the pseudoglobulin fraction of plasma by the action of plasmin, a proteolytic enzyme. Plasmin is present in the blood in an inactive form, plasminogen, which may be transformed into the active enzyme by different activators, such as urokinase, streptokinase, and kallikrein (11, 113, 144).

Pain-producing substance (PPS) develops when fresh human plasma is brought into contact with glass. Its name originated from the fact that, besides contracting smooth muscles, PPS produces pain when applied to the exposed base of a blister induced by a cantharidin plaster (7, 8, 126). The activation of plasma by contact with glass is by no means a general phenomenon; dog and sheep plasma, for example, are not activated by contact with glass (88). The intimate, rather complicated mechanism of PPS formation as well as the relation of PPS formation to blood clotting have been thoroughly studied by Margolis (148), to whom reference should be made for details.

Urinary kinin (Substance Z) is closely allied or identical to both kallidin and bradykinin. The rate of kinin excretion in human urine varied between 6 and 18 units/min., or, in terms of pure ox bradykinin, approximately 10 to 30 μg./24 hr. (87, 114). The bradykinin-like substance found in human amniotic liquid is probably attributable to fetal urine (202).

Colostrokinins are produced on the incubation of bovine colostrum with urinary kallikrein (urinecolostrokinin) or calf saliva (salivacolostrokinin). No consistent differences were found in parallel assays between colostrokinins and plasma kinin (96). A colostrokinin (serumcolostrokinin) also appears in a mixture of colostrum with serum. At present it cannot be decided whether it is different from bradykinin or kallidin, as suggested by Werle (210).

Once formed, all the bradykinin-like polypeptides undergo, if left in contact with plasma or lymph, rapid inactivation which is caused by a powerful bradykinin-destroying enzyme present in the globulin fraction of the plasma (99). In this regard guinea pig plasma is a particularly potent bradykinin inactivator (173). Bradykinin inactivation in plasma is usually blocked, in the preparation of the polypeptide, by addition of boiling ethanol.

The first question to be posed at this point is: are all kinins identical or not? The bulk of the available experimental evidence points to the identity or close similarity among the various above-mentioned bradykinin-like substances. In fact (*a*) in parallel pharmacological tests, kallidin, bradykinin,

plasma kinin, and PPS were qualitatively and quantitatively indistinguishable in their actions on smooth muscles, blood pressure, and capillary permeability; (*b*) the similarity of all the above bradykinin-like substances extends to their rate of inactivation by plasma and by lymph of various animals as well as to their chemical and physico-chemical properties (166, 173); (*c*) it seems not unlikely that the release of all bradykinin-like substances found in plasma is attributable to the activation of one and the same enzyme, kallikrein.

Elliott, Lewis & Horton (62) in England and Zuber & Jaques (224) in Switzerland have recently succeeded in isolating pure bradykinin from ox plasma incubated with trypsin (bradykinin T) or with *Bothrops jararaca* venom (bradykinin B). The two bradykinins appeared to be identical. On acid hydrolysis bradykinin T yielded serine, glycine, proline, phenylalanine, and arginine in the molar proportion of 1:1:3:2:2. As already suspected by Rocha e Silva (166), the terminal (N-)residue was arginine. It is evident that the contribution of Elliott and co-workers will be of decisive importance in many respects and that it will allow us in the near future to solve the problem of the identity of the different bradykinin-like substances.

Boissonnas and co-workers (27b, 27c) have completed the work of Elliott and co-workers by the synthesis of six peptides related to bradykinin, one of which, the nonapeptide Arg. Pro. Pro. Gly. Phe. Ser. Pro. Phe. Arg., proved to be identical, under every respect, to natural bradykinin T.

Physiological significance.—Bradykinin-like substances have been considered by some research workers to play an important, if not essential role, in functional vasodilatation, i.e., in the local adjustment of blood flow in relation to activity of tissues, especially glands and muscles. In addition, it has been suggested that they participate in the systemic reactions to some drugs as well as in the vascular and sensory phenomena of inflammatory reaction and are are associated with heightened neural activity. These points will be briefly discussed.

(*a*) Functional vasodilatation: Hilton & Lewis (108, 109, 110) are of the opinion that salivary kalikrein acts as a regulator of blood flow to the salivary glands during salivation by releasing bradykinin from plasma. Thus, this vasodilator polypeptide would have an important physiological role as the agent mainly responsible for the functional hyperemia in the glands.

Similarly, in sweat glands and in the pancreas the bradykinin-like substances formed by the action of the sweat gland kinin-forming mechanism or by pancreatic kallikrein could fully account for the active dilatation of the pancreatic vessels during pancreatic secretion and for the dilatation of the vessels of the human skin which occurs when the body is heated sufficiently to cause sweating (44, 81).

Meager experimental evidence supports, up to the present, the suggestion that bradykin-like polypeptides may be ranked among the chemical

agents responsible for the functional vasodilatation in muscles and in the central nervous system and for the local adjustment of renal blood flow (108, 114).

(*b*) Systemic vascular reactions to drugs: Liberation of bradykinin into the general circulation has been suggested as playing a role in peptone shock (15) and, in reserpine hypotension (168), to be involved in the generalized muscle vasodilatation which develops in man and animals during intravenous infusion of epinephrine and norepinephine (110) and, finally, to interfere with the vasodilatation and sweating occurring in the human forearm during insulin hypoglycemia (3).

(c) Inflammatory reaction: Keele and co-workers (7, 8, 126) first demonstrated that plasma can be activated *in vitro* to produce a substance which induces many of the vascular and sensory features of the inflammatory reaction when applied topically to the exposed area of a cantharidin blister. They further showed that a similar substance was present in the blister fluid after burns and in the joint fluid of rheumatic patients (8). These observations were confirmed and extended by other investigators (44, 51b, 102). Having found that a bradykinin-like material is released in the perfusate of human skin submitted to painful stimuli, Harpman and co-workers (102) suggest that a protease is liberated locally immediately following noxious stimulation and that this protease acts on a globulin to form polypeptides that increase permeability, lower pain threshold, and induce vasodilatation, edema, and other reactions relevant to inflammation. It is possible that every trauma leading to activation of proteolytic enzymes in the blood may also release bradykinin (167). Finally, bradykinin could be also one of the "slow reacting substances" released in anaphylaxis (28c).

Bradykinin and central nervous system.—Two groups of research workers have recently pointed to the possibility that bradykinin-like substances may be in some way related not only to peripheral but even to central neuronal activity.

Chapman and co-workers (44) observed an increased bradykinin-forming enzyme activity in cerebrospinal fluid collected from human subjects with inflammatory or degenerative diseases of the central nervous system or in cases of migraine or chronic schizophrenia. Increased amounts of bradykinin-forming enzyme were also found in the cerebrospinal fluid removed from the cisterna magna of cats following induced convulsions. Apparently the bradykinin-forming enzyme in the cerebrospinal fluid is not plasmin, and it has been hypothesized that it may be a neural intracellular proteinase.

Rocha e Silva and his co-workers (167, 168) found that both intracarotid and intraventricular injections of bradykinin produced, besides a fall in blood pressure and relaxation of the nictitating membrane, tranquillization or, the opposite, restlessness with convulsions. However, it should be noted that, at variance with Chapman and co-workers, Hamberg (100) failed to find in the cerebrospinal fluid from patients with central nervous system disease any protease activity sufficient to release bradykinin.

It is very difficult at present to draw any definite conclusion about the physiological or physiopathological role of bradykinin-like polypeptides. However, if the pessimistic statement of Schachter (173) that "there is as yet no convincing evidence of any specific physiological function of bradykinin" seems in its formulation somewhat crude, noboby can deny that it is substantially true. The main objection which may be raised in regard to the work dealing with the physiological role of bradykinin is that results obtained under particular experimental conditions or in single animal species have too often been unduly generalized. It is all too easy to encounter experimental evidence which does not fit in with general hypotheses and theories.

Pharmacological actions.—These have been described in detail for many years. Hence, only a brief account of some more recent contributions will be given here. The most important of the pharmacological actions is certainly that of Elliott and co-workers (62) who found that pure bradykinin from ox blood was active on a number of preparations and that within the errors of biological variation the increase in activity from the crude material was proportional in all tests. This means that all the activities of crude bradykinin preparations are inherent properties of the pure polypeptide, and this validates at once all the preceding data on the pharmacological actions of crude bradykinin preparations. It was shown that the active concentrations of the pure polypeptide varied between 0.0002 μg./ml. required to contract the rat uterus and 0.4 μg./kg. required to cause a depressor response in cats.

The above results, obtained with bradykinin T(trypsin bradykinin), were confirmed by Jaques & Meier (115b) who used bradykinin B (*Bothrops* bradykinin). Jaques & Meier further found that bradykinin B had no action on the blood pressure of the cock in doses up to 3 μg./kg., and that 0.01 to 0.03 μg. of the substance given intracutaneously in guinea pigs increased capillary permeability, as shown by extravascular leakage of circulating dye.

As already stated, Boissonnas and co-workers (27b, 27c) prepared by synthesis six polypeptides bearing strict resemblance to natural bradykinin, more precisely, a nonapeptide, four octapeptides, and one esapeptide. Two of these synthetic peptides, and especially the nonapeptide appeared to possess an intense biological activity. In fact, the guinea pig ileum and the rabbit duodenum were contracted by concentrations of the nonapeptide as low as 0.001 μg./ml., and 0.001 μg. was sufficient to lower the permeability of skin capillaries in the guinea pig. To produce a fall of blood pressure in rabbits, rats, guinea pigs, cats, and dogs, as well as a bronchoconstriction in guinea pigs, the dose ranged from 0.2 to 0.4 μg./kg. Removal of the prolyl residue in position 3 produced a 100-fold decrease in the biological activity; removal of the prolyl residue in position 7, a practically complete disappearance of activity (155b, 180b). Once again it should be stressed that the synthetic nonapeptide is in all probability identical with the natural bradykinin isolated by Elliott.

Some synthetic peptides with only slight structural resemblance to natural bradykinin were studied by Tritsch & Woolley (198). They possessed a negligible bradykinin-like activity.

Another important contribution to the pharmacology of bradykinin is credited to Rocha e Silva and co-workers (167, 168), who observed that the vasodilator effects of bradykinin were strongly potentiated by sympatholytic drugs as well as by some centrally acting hypotensive drugs, such as apresoline, chlorpromazine, and reserpine. Although most of the potentiation observed might be explained on the basis that the return of the blood pressure to normal level was impaired by peripheral sympatholysis or by depletion of the stores of catecholamines in their peripheral stocks, it is possible that in addition some central component participates in the potentiation by apresoline and reserpine. Since many of the effects produced by reserpine have also been observed by intraventricular injection of bradykinin, it is suggested that bradykinin too, should be considered seriously as a possible mediator of the actions of reserpine. Finally, the recent experimental results of Collier and co-workers (49b) should not be passed over in silence. These investigators demonstrated that bradykinin is a potent bronchoconstrictor agent in the guinea pig (at least as potent as histamine weight for weight) and that bradykinin bronchoconstriction is antagonized by acetylsalicylic acid in doses (minimum 2 mg./kg., intravenously) which give concentrations in blood that are much lower than those reached in man during salicylate therapy.

The above observations raise the questions whether bradykinin or a related polypeptide may participate in human asthma and whether suppression of response to bradykinin or to a related polypeptide contributes to the antirheumatic action of acetylsalicylic acid and other nonsteroid agents.

Bioassay of bradykinin-like substances is usually carried out on the blood pressure of cats and rabbits, on guinea pig and rabbit ileum, and on the duodenum and uterus of the rat. To these preparations the intestine of cats and dogs may be profitably added. Both preparations are fairly sensitive and show an excellent dose-response relationship (Erspamer, unpublished observations).

Substance P

Substance P is a polypeptide of unknown chemical structure and amino acid composition first described by Euler & Gaddum (73) in extracts of many tissues. The purest known preparation of substance P contains about 3000 units per mg. (161).

Distribution.—Substance P has been found in large amounts in gastrointestinal tract, retina, brain, posterior roots of spinal nerves, and preganglionic sympathetic nerves. Some activity was also detected in many other nerves (6, 58, 86). It is now generally accepted that the substance P in brain and nerves is the same substance as the substance P in intestine (58, 86).

In the alimentary canal maximum concentrations of substance P were found in the duodenum and jejunum of all species. The absolute values, however, greatly varied according to animal species, being greatest in the monkey and smallest in the pig intestine. Stomach contained very little substance P; the rectum, on the contrary, fairly large amounts (58).

Substance P seems to be present in all parts of the central nervous system, but its concentration in the brain varied over a range of more than 100-fold. The highest concentrations were found in human substantia nigra (700 units/gm.), in the ala cinerea, area postrema, central midbrain, hypothalamus, and caudate nucleus; the lowest in the cortex and cerebellum. There are wide differences in the concentration of substance P in different brains of the same species (58, 220). Attention has been repeatedly called to the fact that large amounts of substance P are contained in the dorsal roots, the posterior columns of the spinal cord, and the nuclei gracilis and cuneatus, structures all containing the same actual nerve fibers and little or no acetylcholine or choline acetylase (58, 136).

Substance P is not confined to mammals; it has been found in the intestine of fishes and even ascidians and in the central nervous system of all vertebrates (52, 54). The distribution of substance P in different areas of the central nervous system of the dogfish was the same as in mammals (54).

Enormous amounts of substance P were found in the brain of amphibians (up to 500 to 1000 units/mg.) (52, 93), but it is doubtful whether in this case all the activity on atropinized guinea pig ileum is actually caused by substance P. Other slow-reacting substances of unknown nature probably contribute to the stimulation of the gut. Following fractionated centrifugation of cat brain homogenates, 80 per cent of substance P was found in the sediment, especially, in the mitochondrial fraction (138).

Pharmacological actions.—For the actions of substance P on blood vessels and on extravascular smooth muscles we refer to the review articles of Pernow (160b, 161). Here it should only be remembered that there is as yet no clear understanding of the mechanism of action of the polypeptide on the intestinal muscle. The opinion of Pernow that substance P acts on two different sites—directly, by stimulating the smooth muscle fibers and, indirectly, by stimulating the nervous elements of the intramural reflex arc—is not completely shared by Kosterlitz (133) who could not find unequivocal evidence that the muscular contraction caused by the polypeptide was attributable to stimulation of nervous structures.

Since primary interest has recently been centered on the actions of substance P on peripheral nervous structures and central nervous system, these points will be discussed in some detail here.

(*a*) Action of substance P on peripheral nervous structures: Beleslin and co-workers (14) found that intraarterial injections of substance P in doses from 10 to 30 units potentiated the response of the nictitating membrane to submaximal stimulation of the sympathetic preganglionic nerve,

whereas higher doses usually depressed the response. The stimulatory actions of acetylcholine on the superior cervical ganglion and of epinephrine, norepinephrine, and tyramine on the nictitating membrane were also potentiated by the polypeptide.

(*b*) Central actions of substance P injected into the general circulation: Substance P injected subcutaneously in mice, in doses of 500 to 3000 units/kg., caused an inhibition of spontaneous movements and sedation. This effect was also obvious in animals pretreated with psychomotor stimulants, such as morphine, methamphetamine, and amino-β,β-dipropionitril (191, 223). Similarly, a definite, long-lasting taming effect has been observed in wild hares given intravenously very low doses of the polypeptide (100 to 300 units/kg.) (192). At rather high dose levels (8000 to 16,000 units/kg., subcutaneously) substance P was further found to potentiate in mice barbiturate hypnosis and bulbocapnine catatonia, to antagonize harmine tremors as well as the convulsant activity of strychnine, picrotoxin, and tetanus toxin (not that of caffeine, nicotine, ammonium salts, or electroshock), finally, to produce hyperalgesia, and to counteract morphine analgesia (222, 223). In guinea pigs, substance P (12,000 units/kg., subcutaneously) at first also antagonized the morphine-induced depression of the respiratory center, but later it deepened morphine paralysis. In untreated guinea pigs, substance P alone displayed a clear depressant effect on respiration (223).

Not all the above results of Zetler could be confirmed. In fact, Stern & Hukovic (194) found that whereas crude preparations of substance P possessed exactly the actions described by Zetler, purified substance P (270 units/mg.), given intraperitoneally in doses of 1000 to 10,000 units/kg. did not protect the mouse against strychnine convulsions nor did they prolong evipan narcosis. Similarly, whereas small doses (1000 units/kg.) of purified substance P caused, like unpurified preparations, hyperalgesia, high doses (10,000 units/kg.) did not produce this effect. It is evident that if the above observations of Stern & Hukovic are correct, the whole problem of the central effects of substance P should be re-examined with pure polypeptide.

(*c*) Central effects of substance P injected into the cerebral ventricles: Following intracisternal or intraventricular injection of 5 to 50 units substance P in cats or rabbits, a variety of symptoms made their appearance, the most constant autonomic effect being a long-lasting stimulation of respiration, and the most marked behavioral change a general inhibition of spontaneous activity (76).

(*d*) Action of intracarotid substance P on cortical and hippocampal EEG: Intracarotid injections of 30 to 100 units substance P in rabbits caused a decrease in amplitude and an increase in frequency in the cortex, and a synchronization of the hippocampal activity. It was concluded that the ascending activating system of the reticular formation was stimulated by the polypeptide (135).

(*e*) Action of local, epicortical application of substance P on the elec-

trical activity of the rat brain: The local application of 4 to 10 units substance P on the rat cortex produced a displacement of the zero potential of the cortex to the positive side and increased the dendrite potentials. These effects were eliminated by cathode polarization and by γ-aminobutyric acid (40).

(*f*) Correlation between reserpine sedation and substance P: Having observed that reserpine provokes an increase in the substance P concentration of the brain of rats (from 14 to 19 to 70 units/gm.) and rabbits, Stern & Kocic-Mitrovic (193) suggest that the tranquillizing action of reserpine may partly depend upon the rise in the level of substance P which is considered a "physiological tranquillizer." Unfortunately, the results of the Yugoslav investigators are at variance with those of Paasonen & Vogt (157) who failed to find any influence of reserpine and various other drugs on the substance P concentration in brain.

At this stage it may be said that, in spite of all the above experimental efforts, the assumption that exogenous substance P exerts distinct direct effects upon the central nervous system still lacks any satisfactory experimental basis. The main objection which should be raised against the validity of all or nearly all the above experiments is that the investigators have completely overlooked the powerful vasodilator action possessed by substance P. Doses of up to 8000 to 16,000 units/kg. were administered parenterally without any apparent control of systemic blood pressure and of temperature. Yet, in human beings the infusion of quantities of substance P as low as 30 to 50 units/min. (i.e., 0.5 to 1 unit/kg./min.!) was sufficient to bring about a bright red flush in the head immediately following the start of the infusion (56b, 146), and in dogs intravenous doses of substance P as low as 0.2 to 0.5 unit/kg. were capable of producing a fall of blood pressure of 40 to 60 mm. Hg (Erspamer, unpublished observations).

Although results obtained in humans and dogs cannot be generalized to other animal species without adequate experimental support, it seems imperative to exclude, before speaking of true central effects of substance P, any interference by systemic or local vasomotor changes produced by the polypeptide.

Physiological significance.—(*a*) Intestinal substance P: It has been suggested that substance P in the alimentary canal is associated with the intramural nervous plexuses and that it controls or participates in the control of the activity of the intestinal smooth muscle. More precisely, substance P would produce, like 5-HT, a reflex contraction of the longitudinal muscle coat by stimulation of sensory nerve endings (13). Without constituting definite evidence, some facts support this view. Substance P is a powerful stimulant of intestinal motility in numerous animal species both when given by intravenous route and intraluminally (13, 89). In man a marked increase in the segmental and peristaltic movements of the small intestine was obtained following intravenous infusion of substance P in doses as low as 50 units/min. (146). Moreover, Ehrenpreis & Pernow (57)

found low concentrations of substance P in the inactive, aganglionic part of the gut in Hirschsprung's disease and abnormally large amounts in the hyperactive gut above this point.

(*b*) Substance P in peripheral nerves and dorsal roots: Since substance P is present in large amounts in the posterior roots of spinal nerves and in the dorsal columns of the spinal cord, structures which contain little or no acetylcholine or choline acetylase, it has been suggested by Lembeck (136) that the polypeptide might be the chemical transmitter liberated by the first sensory neurone. The opinion has also been advanced by Holton (112) that substance P might be associated with the transmission of nervous impulses from nerve endings. This view was mainly based on the assumption that substance P was formed in the nerve cells of the spinal ganglia and moved down the axon to nerve endings. However, in more recent experiments, Holton (111) found that after dividing the sciatic nerve of rabbits, the distal end of degenerating nerve contained residual substance P varying in different animals from 5 to 73 per cent of that in the control nerve. Obviously, this important fraction of substance P could not be concerned with normal nervous activity.

(*c*) Substance P in the central nervous system: The pharmacological properties of substance P and its prevalent distribution in brain areas which contain relatively low concentrations of choline acetylase led Kopera & Lazarini (132) to the view that substance P is a noncholinergic transmitter in the central nervous system. A similar opinion is shared by Zetler (223) who considers the polypeptide as an inhibitory transmitter, and by Stern & Milin (192), who consider it a "physiological tranquillizer," displaying its depressive action mainly on the hypothalamus. Actually, as Zetler admits, all suggestions about a possible transmitter function of substance P are merely hypothetical.

To conclude, we can only say at present that substance P should have some function in the tissues in which it is formed. What this function is, and how important, is largely obscure. This is particularly true for the substance P present in the brain.

Other Active Polypeptides

Angiotensin, substance P, and bradykinin-like substances are the main representatives of the pharmacologically active polypeptides. However, several other active substances belonging to this group have been described. Since these additional polypeptides are as yet poorly defined and characterized or appear of doubtful biological importance, they will not be discussed here in detail.

Active polypeptides formed in serum by pepsin and by acidification.— The hydrolysis of blood serum with pepsin produces the liberation of substances with conspicuous pharmacological effects resembling those of angiotensin and the hormones of the neurohypophysis. These substances are

pepsitensin, possessing vasoconstrictor effects similar to those of angiotensin; pepsitocin, producing a tonic contraction of the atropinized rat and guinea pig uterus; and pepsanurin, causing a powerful antidiuretic action. The problem whether these substances are merely artifacts or whether they have some role in homeostasis is still open, as is the problem of their individuality (53b). The same may be said for the oxitocic and pressor polypeptide originated in the serum from the globulin fraction by acidification (53c).

Substance A.—The incubation of fraction IV-4 of plasma protein with α-amilase produces the appearance of a hypertensive smooth-muscle-stimulating polypeptide. From a pharmacological point of view, substance A is very similar, if not identical, to angiotensin (205b).

Hysterotonin.—A pressor substance found in highest concentration in decidua and amniotic fluid in patients with toxemia was given the name hysterotonin by Hunter & Howard (114b). This substance seems to be a polypeptide formed by an enzymatic reaction. It is claimed that it differs from every other previously described polypeptide.

Vasodilator and spasmolytic polypeptide of lymphoglandular origin.—This factor has been found by Vallette and co-workers (201b) in the hydrolysate obtained by controlled action of papain on ox lymphatic ganglia.

Pharmacologically active polypeptides are now at the center of enormous interest and fervid biochemical and pharmacological research. We are dealing with a group of compounds of relatively simple character (their molecular weight falls generally between 1000 and 2000) which possess chemical structures that have already been established or are near being elucidated, and which produce tremendously intense pharmacological effects bearing comparison with those of the most powerful neurohumoral amines. Some of the pharmacologically active polypeptides have already been reproduced by synthesis, thus permitting extensive and profound research with absolutely pure compounds.

Recent biochemical and pharmacological development will certainly help, in a decisive way, to solve the central problem in the investigation concerning the active polypeptides, that of their particular significance in physiology and pathology.

LIPID-SOLUBLE ORGANIC ACIDS

Prostaglandin (59, 203) is a mixture of lipid-soluble organic acids found in the vesicular glands (not in the prostate!) of sheep and in the semen of sheep, goat, and man. The substance present in the seminal fluid from man is secreted exclusively from the seminal vesicles.

Prostaglandin is present in the tissue in an inactive form and is secreted into the seminal fluid in a free, active state. Under appropriate conditions of incubation, the amount of free active prostaglandin in sheep's ground

vesicular glands can be increased by 40 times. The human seminal vesicles are capable, in some cases, of replenishing their content of prostaglandin within 14 to 24 hours.

Prostaglandin stimulates both *in vitro* and *in vivo* the intestine and uterus of various species and produces a long-lasting fall in arterial blood pressure. However, both tonus and spontaneous activity of the isolated nonpregnant human uterus are depressed by the substance.

Recently, two crystalline compounds, both nitrogen-free, unsaturated acids, have been isolated from the vesicular glands of sheep (16). Characteristic differences in the activity ratio of the two crystalline fractions, named Prostaglandin E and Prostaglandin F, were noted on different organs. Prostaglandin E, for example, lowered the rabbit blood pressure in doses from 0.5 μg., whereas no action was observed with prostaglandin E in doses up to 10 μg. The approximate threshold dose of both prostaglandin E and prostaglandin F on the rabbit jejunum (a preparation which is frequently used in the bioassay of prostaglandin) is 0.003 to 0.01 μg. per ml. nutrient liquid (17).

Chemically pure prostaglandin E infused in doses of 0.2 to 0.7 μg./kg./min. in healthy human subjects over periods of 4 to 10 min. produced tachycardia, reddening of the face, headache, and an oppressive feeling in the chest. Systemic arterial blood pressure and cardiac output fell only moderately (18).

As already stated, it is probable that prostaglandin is not an unitary substance but a mixture of various related compounds. This opinion is corroborated not only by the isolation of prostaglandin E and prostaglandin F, but also by the fact that the biological actions of partially purified preparations of prostaglandin from the sheep vesicular glands could be largely, but not wholly, explained by assuming that they contained a mixture of prostaglandin E and prostaglandin F (17, 59).

Prostaglandin does not seem to have a general biological significance since it is absent in the seminal fluid and male accessory genital glands of a number of mammals (stallion, bull, boar, dog, cat, rabbit, guinea pig). It has been suggested that, in human beings and sheep, prostaglandin may promote ejaculation by inducing peristalsis of the male genital tract, may stimulate the motility of the spermatozoa, and may facilitate the migration of spermatozoa in the female genital tract (17). Of the three suggestions the last seems to be less hypothetical.

An acid substance present in the semen and in the vesicular glands and the prostate of the monkey, vesiglandin resembles prostaglandin in many respects but differs from it in having a relatively stronger hypotensive action and a weaker muscle-stimulating effect (74).

Darmstoff is an active principle extracted by Vogt (203) from bath fluids in which a piece of intestine had been suspended or from intestinal tissue itself. In the intestinal wall most of the substance appears to be bound and to become easily extractable only after the disintegration of the tissue.

The properties of darmstoff are those of a lipid-soluble acid, and it has recently been characterized as a mixture of closely related and, as yet, unseparated acidic phospholipids. It is possible that the major component of the mixture is an acetalphosphatidic acid.

Darmstoff increases the tone of the longitudinal muscle of the intestine of all species investigated, including those which do not react to histamine. The isolated rabbit duodenum is particularly sensitive (threshold concentration for purified preparations is less than 0.001 μg./ml.) and widely used in the bioassay of the substance. The isolated uterus and the urinary bladder are also contracted. The mechanism of action of darmstoff on the gut is not completely understood and it may be different in different species. Vogt believes that the substance does not act directly on the smooth muscle fibers: a noncholinergic action being predominant in the rabbit intestine and a cholinergic, but not necessarily neuronal, action predominating in the guinea pig ileum. Darmstoff does not produce any remarkable effect on the circulatory system.

On the basis of its occurrence and action, Vogt suggests that darmstoff plays a physiological role in the motor activity of the gut and, more precisely, that it is the postganglionic transmitter for atropine-resistant vagal impulses. Thus, lipid-soluble acids also may participate, together with biogenic amines and polypeptides in the control of intestinal motility.

Watery extracts of rabbit iris contain an unsaturated lactonizing hydroxy-fatty acid, irin, which is capable of contracting iridial and intestinal smooth muscles (4). The histamine-insensitive rat or hamster colon preparation is used for the routine estimation of irin; it is capable of detecting the activity corresponding to 0.25 to 1 mg. of iris per ml. organ bath (equalling 0.1 to 1 μg. purified irin). On the isolated ox iris preparations, doses of purified irin as small as 0.3 to 1 μg./ml. produce a strong and long-lasting contraction of the sphincter muscle. Irin-like activity is present not only in iris tissue, but also in aqueous humor, in the conjunctiva, in trigeminal nerve, ventral and dorsal ear skin, and muscularis externa of the intestine.

So far irin has not been obtained in a pure form. However, watery crude extracts have been remarkably purified through ether, acetone plus chloroform extraction, and electrophoresis. Irin possibly plays a role in the function of the iris and since the substance is occasionally released into the aqueous humor after stimulation of the trigeminal nerve, Ambache (4) suggests that it is the transmitter for the atropine-resistant miosis which occurs on antidromic stimulation of the trigeminal nerve.

G-Acid is a straight-chain, unsaturated monocarboxylic acid, probably identical with 3-octadecenoic acid, which has been isolated by Gabr (84) from the G2 fraction of human plasma. G-Acid, which appears to be preformed in plasma or loosely bound, produces a slow and delayed contraction of the isolated guinea pig and rabbit jejunum, possesses a marked hemolytic action on human red cells, and displays a vasoconstrictor activity on rabbit

coronary arteries. The biological significance of the acid is completely obscure.

One of the hemolytic agents occurring in blood and tissue, especially brain, has been characterized by Laser (134) as an unsaturated monocarboxylic acid, possibly the *cis*-11-octadecenoic acid (*cis*-vaccenic acid). The compound possesses strong hemolytic effects and causes a delayed contraction of the guinea pig ileum. The function of *cis*-vaccenic acid in brain is completely obscure. It has been suggested that the acid occurring in red cells may have a bearing in the physiological destruction of aged erythrocytes.

Arachidonic acid, a highly unsaturated fatty acid normally present in body lipids, possesses a powerful smooth-muscle-stimulating effect and causes pain when applied to human skin, in concentrations as low as 3 to 30 μg./ml. According to Jaques (115) this acid, too, may play some physiopathological role.

The so-called slow-reacting substance A is an acid lipid-soluble, smooth-muscle-stimulating principle which appears, together with histamine, in the venous effluent of perfused sensitized guinea pig, rabbit, or monkey lungs on injection of the antigen (29), and in the incubation fluid of tissues from sensitized guinea pigs on incubation with the antigen (42). It was also possible to recover the same, or a closely related principle, from the perfused cat paw after the injection of compound 48/80 (43). No slow-reacting substance A or histamine was detected in the effluent from perfused lungs of rats on the addition of antigen (29).

Since on the one side there is a close correlation between the amounts of histamine and slow-reacting substance A obtained from different tissues, and, on the other, a good proportionality between the content of the two substances and the mast cell population, it has been suggested that the tissue mast cells may be the common source of both histamine and slow-reacting substance A (28b). According to Brocklehurst (29), its formation occurs chiefly in lung and vascular tissue. The isolated atropinized guinea pig ileum is the preparation most suitable for the bioassay of slow-reacting substance A.

It is highly probable that this substance contributes to the symptoms of anaphylaxis, and it is possible that it may also be of significance in human asthma. In fact, Brocklehurst found that it is formed by the action of allergen in human asthmatic lung and that it produces bronchospasm in man (29).

Other substances possibly related to lipid-soluble acids are fraction X found, like darmstoff, in intestinal extracts (203); eutocine, occurring in uterine muscle tissue and amniotic fluid of man at the time of delivery (101); the plain muscle stimulant principle present in the menstruum (162); and pressor or smooth muscle contracting substances found in the skin (5, 145) and brain (221).

As pointed out by Vogt, who has recently written an excellent review article on the subject (203), the lipid-soluble organic acids have attracted

general attention only in the last few years. In fact, it is only since modern analytical and preparative methods have become available that considerable progress has been made in the study of these substances. It is quite probable that the list of the compounds discussed in the present paper is destined to lengthen in the near future, and it is hoped that future research will help to elucidate the many obscure points concerning biosynthesis, liberation, fate, and, above all, biological significance of the pharmacologically active lipid-soluble acids occurring in the mammalian organism.

It is evident, however, that the necessary requirements for any progress in this field will be the exact characterization and individuation of the different compounds. It is probable that very often we have to deal with mixtures instead of with individual substances (prostaglandin, darmstoff, etc.), and one cannot avoid the impression that not infrequently the same activity has been attributed to different compounds by different investigators.

Generally, lipid-soluble acids are considered local hormones, but their distribution is often difficult to reconcile with the idea of a specific local function (a darmstoff-like substance occurs in skeletal muscle and in brain; irin-like activity occurs in the skin and in the intestinal wall, etc.). Vogt rightly believes that they may have a more general function. Lipid-soluble acids are substances with the general property of stimulating smooth muscles. Since stimulation of excitable cells is brought about by depolarization, i.e., by equilibration of concentration differences of cations inside and outside the cell, Vogt suggests that it is conceivable that acids capable of forming complexes with cations might act as carriers of negative charges in the cell membrane, thus interfering with both the excitability and the excitation of the cell.

CONCLUSION

The three groups of active substances discussed in the present paper, while showing decisive differences in their chemical structure, share important pharmacological properties. In fact, all possess powerful actions on vascular and extra-vascular smooth muscles.

The kindred pharmacological character of amines, polypeptides, and lipid-soluble acids emphasizes the opportunity of considering all three groups of compounds when trying to interpret the action of crude tissue extracts or explaining the pathogenesis of physiological and pathological processes involving changes in caliber or permeability of blood vessels, changes in tone and motility of extra-vascular smooth muscles, or both. It may be that representatives of the three groups of substances are liberated contemporaneously or successively in the same physiopathological process or following the administration of the same pharmacological agent.

The antigen-antibody reaction, for example, may set free not only histamine and 5-hydroxytryptamine, but also bradykinin and the slow-reacting substance A; reserpine, in its turn, has been claimed to liberate not only indolealkylamines, catecholamines, and histamine, but also bradykinin, and to increase the brain concentration of substance P.

These elementary concepts and facts are very often overlooked.

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PHARMACOLOGY OF AUTONOMIC GANGLIA[1,2,3]

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This report is a review of the work on autonomic ganglion cells carried out during the last three years. Since this is the first volume of a new series of *Annual Reviews,* pertinent older literature will also be mentioned. The reviewer thought it appropriate to include what may be described as "odd results," *i.e.,* observations which have not yet found an explanation or even confirmation. Such findings may turn out to be artifacts rather than genuine drug effects; they may, however, turn out to be the beginning of a new and important development.

The reviewer does not see any necessity to challenge the classical concept that the release of acetylcholine (ACh) is the central event in ganglionic transmission, although there is good possibility that detailed studies with improved methods of the mechanism of synaptic transmission will lead to some modifications of the classical theory. During the last few years, the following suggestions have been made: (*a*) An inhibitory system arising from the central nervous system (CNS) may act on the presynaptic nerve terminals. (*b*) Presynaptic nerve terminals may be an important site for the action of drugs hitherto believed to have an action on postsynaptic structures only. Similar ideas are discussed in connection with motor nerve endings, and postganglionic nerve endings are now recognized as an important site of drug action. (*c*) Ganglion cells may be affected or even stimulated by a variety of substances which differ from ACh and the well-known group of nicotine-like substances. (*d*) Peripheral ganglion cells may serve functions which extend considerably beyond those of a simple relay station; they may, for instance, be acted upon by sensory structures.

For earlier reviews the reader is referred to those of Paton (168, 169), Ambache (4, 5), and Perry (176), which cover part of this field. Attention is also drawn to Konzet & Rothlin's interesting comparison of the effects of various drugs on autonomic ganglion cells, on the adrenal medulla, and on certain sensory structures (117). Such comparisons are good illustrations of the view that seemingly qualitative differences between various tissues may be, in some respects, quantitative rather than qualitative in nature (see below).

[1] The survey of literature pertaining to this review was concluded in June, 1960.

[2] The following abbreviations have been used in this chapter: ACh (acetylcholine); SCG (superior cervical ganglion); TEA (tetraethylammonium); TMA (tetramethylammonium); DFP (di-isopropylfluorophosphate); TEPP (tetraethylpyrophosphate); CNS (central nervous system).

[3] This work was supported in part by U. S. Public Health Service Research Grant B-1713.

The isolated and perfused superior cervical ganglion (SCG) of the cat is the classical preparation for the study of the pharmacology of autonomic ganglia. From a survey of the literature it is evident that the disadvantages and limitations of this preparation are not always realized; they have been discussed by Paton (168) and by Ambache (4), and important modifications have been described or referred to by Perry (175). An additional disadvantage of perfused preparations is their low sensitivity to certain substances; the perfused ganglion is 100 times less sensitive to histamine and 5-hydroxytryptamine than the nonperfused preparation (222). For details of method of the nonperfused SCG of the cat see (115, 168, 219, 226).

Observations on Anatomy and Physiology of Autonomic Ganglia

The toughness of the tissues surrounding ganglion cells precludes the use of certain electrophysiological methods as routine procedures in the analysis of the action of drugs on ganglionic transmission. In comparison with the study of the neuromuscular junction, the pharmacology of autonomic ganglia is, thus, severely handicapped. Because of the many similarities of the two transmission processes, it is customary to apply certain conclusions from one field to the other by analogy. It should, however, be borne in mind that there are many anatomical differences which may imply functional differences. The preganglionic fibers of the SCG, for instance, consist of four different groups possessing different conduction velocities [S_1 to S_4, Eccles (51)]. The fastest (S_1) innervate structures in the orbit, whereas S_2 fibers carry vasomotor impulses. These observations have recently been confirmed by Folkow *et al.* (65), who also identified the very slowly conducting fibers as sympathetic vasodilators. To the reviewer's knowledge no systematic study has yet been made of the stimulating or depressing action of drugs on these functionally differing groups of fibers. In the evaluation of ganglion-blocking substances it is customary to use either the nictitating membrane (i.e., the S_1 fibers) or "the postganglionic action potential" (presumably the S_2 fibers, since these contribute most to the size of the action potential). It is unknown, however, whether the different groups of fibers are equally sensitive to ganglion-blocking agents; occasionally reported "resistent" pathways (see below) may find their explanation in such quantitative differences of sensitivity.

Douglas & Ritchie (49) reported the occurrence of an accessory cervical ganglion in the rabbit, and Boyd (21) discussed the occurrence of intermediate sympathetic ganglia, which may escape sympathectomy. Chronic preganglionic denervation of the SCG increases its sensitivity to ACh by a factor of about three (38) and completely changes the characteristics of its response to hexamethonium and azamethonium [Perry & Reinert (178)]. Perfusion of a normal ganglion with low-potassium perfusate results in similar changes, whereas perfusion of a denervated ganglion with high-potassium perfusate or with glutamate restores it to normal. Further

analysis of the effects of various amino acids and determinations of intracellular potassium concentrations showed that this "effect of denervation" is attributable to changes in the oxidative metabolism of denervated ganglion cells rather than to their decreased intracellular concentration of potassium ions (77, 179).

Homologous reinnervation (i.e., reinnervation of the SCG by cholinergic fibers of the glossopharyngeal, the phrenic, or the vagus nerve) is easily established. Contrary to earlier postulates, heterogeneous reinnervation also seems to be possible: de Castro (37) reported that the ascending sensory fibers of the vagus are able to make functional contact with the ganglion as indicated by a response of the nictitating membrane to stimulation of the afferent fibers of the vagus. Unfortunately, this interesting "synapse" has not been studied pharmacologically. Sensory vagal fibers can be assumed to liberate the sensory transmitter substance; Holton recently confirmed earlier observations that antidromic stimulation of sensory nerves of the rabbit ear causes the appearance of ATP in the perfusate (99). The occurrence and importance of collateral sprouting after partial denervation of the SCG has been studied in great detail by Murray & Thompson (157, 158).

Zakusov & Ul'ianova (245) observed that distension of the bladder or of part of the intestine causes a relaxation of another part of the intestine. This viscero-visceral reflex persists after total destruction of the CNS and after chronic preganglionic denervation; it is abolished by ganglionectomy or by ganglion-blocking substances. The abnormal pathways described by Brown & Pascoe (24), McLennan & Pascoe (153), and Job & Lundberg (104) may be involved. A somewhat similar situation is encountered in the peristaltic reflex which is well known to be independent of the CNS and to be blocked by hexamethonium. Such observations seem to imply that sensory fibers may establish functional contact with peripheral ganglion cells of the autonomic nervous system. Although ACh is able to stimulate sensory organs and nerve endings, and although hexamethonium abolishes these effects, the latter substance is not able to block the response of sensory structures to their physiological stimulus (50, 83). Such evidence suggests that the site of action of hexamethonium in the above mentioned reflexes is probably at some ganglion cells and not in the afferent pathway of the reflexes.

Considerable evidence is accumulating for the view that presynaptic motor nerve terminals are an important site of drug-action [for review see Werner & Kuperman (238)]. Riker & Szreniawski (186) found that ACh and TMA, when injected into the blood supply of the SCG, elicit antidromic preganglionic activity in addition to the well-known postganglionic effects. Intermittent spontaneous antidromic activity in preganglionic fibers has also been demonstrated in the SCG of the rat after infection with pseudorabies virus, the activity being increased by ACh and physostigmine, and inhibited by *d*-tubocurarine [Dempsher *et al.* (44, 45)]. This activity is increased by topical application of cocaine or by section of the pre-

ganglionic fibers, and reduced by gamma-aminobutyric acid, epinephrine, and norepinephrine (46, 47). From such observations it has been postulated that ACh has a presynaptic site of action and that an inhibitory system originates in the CNS and acts on the preganglionic nerve terminals.

Factor I of Florey & McLennan (63) does not affect synaptic transmission through the SCG of the cat, but other ganglia of the cat and of the rabbit are blocked (63, 100). Gamma-aminobutyric acid, gamma-aminobutyrylcholine, β-guanidinopropionic acid, and gamma-guanidinobutyric acid, on the other hand, are ineffective (100, 147, 217).

The role of cholinesterase.—Karczmar & Koppanyi (108) demonstrated the importance of "transport" cholinesterase; an increased response to injected ACh was obtained by reduction of the activity of plasma cholinesterase when there was no concomitant change of the cholinesterase activity of the effector organ.

Earlier observations by Kamijo & Koelle (107) that inhibition of acetylcholinesterase [by di-isopropylfluorophosphate (DFP)] causes facilitation of ganglionic transmission have been confirmed by Fehér & Bokri (62), who used tetraethylpyrophosphate (TEPP) as an inhibitor. The depressant effects of very large amounts of DFP (107) are, presumably, a direct effect of this substance on postsynaptic structures and unrelated to the inhibition of cholinesterase, since TEPP failed to block ganglionic transmission (62).

In recent years cholinesterase inhibitors that do not penetrate into ganglion cells have become available (111), and with their help it has been possible to demonstrate histochemically that part of the enzyme is located intracellularly ("internal") and part of it extracellularly ("external acetylcholinesterase"). McIsaac & Koelle (151) analyzed the physiological and pharmacological importance of internal and external acetylcholinesterase by using an inhibitor which blocks both enzymes, and another one which blocks only external acetylcholinesterase. Comparison of the effects of such a pair of inhibitors on ganglionic transmission, on total enzyme activity, and (by histochemical methods) on external and internal acetylcholinesterase suggests that only external ("functional") acetylcholinesterase is involved in the hydrolysis of endogenously liberated ACh; internal ("reserve") acetylcholinesterase may serve as a source of replacement of the former. A study of normal and of chronically denervated sympathetic ganglia showed that essentially all the functional cholinesterase is presynaptic, whereas in the ciliary ganglion it is both pre- and postsynaptic. Koelle & Koelle (112) concluded from these and other observations that the primary physiological role of ganglionic functional acetylcholinesterase is the prevention of postsynaptic activation by ACh liberated during rest; a secondary role may be the protection of presynaptic nerve terminals against initiation of antidromic firing by ACh.

Preganglionic (cholinergic) fibers of sympathetic ganglia have a high acetylcholinesterase activity, as demonstrated by histochemical methods, whereas postganglionic (adrenergic) fibers have a low activity (78, 112).

Holmstedt & Sjöqvist (97) found that only a small number of ganglion cells of various sympathetic ganglia was stained, the proportion of stained cells being greater in the SCG and stellate ganglion than in the abdominal ganglia. These observations may be related to the fact that some sympathetic ganglion cells have cholinergic axons (vasodilator fibers, etc.); but whatever explanation may be found, the findings illustrate the inhomogeneity of the cells of sympathetic ganglia.

Ganglion-Stimulating Substances

Nicotine-like substances.—These may be defined as ganglion-stimulating substances, the action of which is *(a)* abolished by depolarization of the ganglion cells as well as by the "competitive" ganglion-blocking substances (hexamethonium, TEA), and *(b)* resistant to atropine administered in amounts sufficient to block "muscarinic" effects of ACh. Schneider & Timms (196) studied the nicotinic, muscarinic, and atropine-like potency of a homologous series of choline esters of fatty acids; muscarinic potency declines beyond ACh, nicotinic potency increases with chain length and declines beyond butyrylcholine, and atropine-like activity is strongest with tetradecanoylcholine. Maleylcholine and succinylcholine cause a rise of blood pressure without prior atropinization, lactylcholine and pyruvylcholine only after atropine; lactylcholine causes a pressor response mainly through an action on the adrenal medulla, pyruvylcholine mainly through stimulation of sympathetic ganglia (54). N-methyl- and N,N-dimethylcarbachol are much weaker muscarine-like agents than carbachol, but their nicotine-like potency is four times greater (8). The absence of any stimulant action of acetyl-β-methylcholine on the adrenal medulla has been confirmed (34), but butyryl-β-methylcholine and benzoyl-β-methylcholine stimulate the superior cervical ganglion (41). Other nicotine-like choline esters are benzoylcholine and certain derivatives (164), nicotinylcholine (but the corresponding bis-quaternary compound is a pure ganglion-blocker) (96), pyridylacetylcholine (191), and a series of thiocholine esters (244).

Murexine [β-(4-imidazole) acrylcholine] like other β-substituted acrylcholines has hardly any muscarinic effects but stimulates ganglion cells (56, 98). In a series of 19 derivatives of murexine, dihydromurexine was the most potent (57). Its ganglionic effects, as well as those of murexine and of imidazoleacetylcholine, have been confirmed and the study extended by other workers (216, 243).

p-Bromo-benzyltrimethylammonium bromide has nicotine-like actions and resembles tetramethylammonium (TMA), whereas the corresponding unsubstituted or *m*-bromo- and *o*-bromo-compounds have only muscarine-like actions (42). A naturally occurring substance, (m-hydroxyphenethyl) trimethylammonium picrate (leptodactyline) is nicotine-like with apparently no muscarinic activity (58). The ganglionic actions of dimethylphenylpiperazine have recently been examined in detail by Ling (133); further studies of the cardiovascular actions of this substance showed that dimethyl-

phenylpiperazine, in addition to its ganglionic actions, elicits reflexes and acts directly on vascular and cardiac muscle (203, 241, 242).

Nonnicotinic ganglion-stimulating substances.—In recent years a study has been made of a group of ganglion-stimulating substances which differ in some respects from nicotine-like agents. Although their effects on ganglia are blocked by "depolarizing" ganglion-blocking substances, they are not antagonized by amounts of "competitive" blocking substances which abolish the effects of nicotine-like substances. Their mode of action has recently been discussed (226), and only a few points will be summarized here.

(a) Histamine.—Small doses facilitate submaximal ganglionic transmission and increase the response of the ganglion to ACh and similar substances (55, 114, 133, 219, 220, 222, 223); moderate doses stimulate the nonperfused SCG, and this effect is antagonized by "depolarizing" ganglion-blocking substances, by cocaine, and by morphine (219, 224, 225) but not by hexamethonium (219). The stimulation of the adrenal medulla by histamine is also not antagonized by hexamethonium or tetraethylammonium (TEA) (87, 219). Large amounts of histamine apparently block the transmission of supramaximal preganglionic impulses through the SCG (75), an observation that does not contradict earlier findings.

The pressor response of the spinal cat to histamine is mainly caused by stimulation of the adrenal medulla (12, 29, 201, 220), but there is also some stimulation of sympathetic ganglia (220). This has been confirmed by the observation that pretreatment with reserpine prevents the pressor response to histamine, whereas 2,6-xylyl ether of choline (which does not influence the release of catecholamines from the adrenal medulla) does not affect it; in adrenalectomized preparations, however, 2,6-xylyl ether of choline (which prevents the liberation of norepinephrine from postganglionic nerve endings) abolishes the pressor response to histamine (227). In rabbits the pressor response to histamine is not mediated through the release of an epinephrine-like substance (162). Neither is the stimulant effect of histamine on isolated atria of various species mediated through the release of an epinephrine-like substance (32, 228), although such a mechanism has been postulated by Went *et al.* (236, 237). Histamine has some actions on the neuromuscular junction (138, 194), and methylated histamine derivatives seem to have more pronounced ganglionic actions (231).

(b) Pilocarpine.—The ganglionic actions of this substance are very similar to those of histamine (199, 219, 220, 222, 223, 226), but, in addition, both its direct actions on smooth muscle and its ganglionic effects are antagonized by very small amounts of atropine (3, 120, 143, 199, 219). Like histamine it causes a pressor response in the spinal cat (and dog); this response is mainly caused by stimulation of sympathetic ganglia, the effect of pilocarpine on the adrenal medulla being rather weak (189, 199, 219). This has been confirmed by the observation that 2,6-xylyl ether of choline and pretreatment with reserpine abolish the pressor response (227).

(c) 5-Hydroxytryptamine.—Stimulating both the superior cervical gan-

glion and the adrenal medulla of the cat, 5-hydroxytryptamine's mode of action resembles that of histamine (55, 187, 221, 222, 223, 235); it seems to block transmission through the ciliary ganglion of the dog (165).

No 5-hydroxytryptamine has been found in ganglia or in the adrenal medulla (68, 72), although the ganglion is rich in 5-hydroxytryptophane-decarboxylase (66). None has been detected in the effluent of the perfused SCG whether resting or preganglionically stimulated. Some 5-hydroxytryptamine appears in the effluent after the addition of amine oxidase inhibitors to the perfusate, but its appearance is not related to preganglionic stimulation (76).

5-Hydroxytryptamine has a stimulant effect on cholinergic nervous structures of the isolated guinea-pig ileum (67, 69, 188) and may also stimulate adrenergic nervous structures of the gut (215). Its stimulant effect on isolated rabbit atria seems to be mediated through the release of an epinephrine-like substance, but it stimulates isolated cat atria by a different mechanism of action (228).

The evidence obtained with these nonnicotinic ganglion-stimulating substances is compatible with the view that their site of action differs from that of "nicotine-like" substances, *i.e.*, that they combine with receptors different from the ACh receptors (226).

Muscarine.—According to classical concepts this substance would not be expected to act on ganglion cells, but, in fact, highly purified preparations (6) and even crystalline muscarine (119) stimulate the perfused SCG of the cat; this effect seems to be abolished by small amounts of atropine (6). In experiments with intravenous injections of muscarine into nonatropinized, or with intra-arterial injections into atropinized preparations, no ganglion-stimulating effects of muscarine have been observed (86, 88, 234), whereas various muscarones had ganglion-stimulating properties under these conditions (88, 234). It seems likely that the very pronounced direct effects of muscarine on smooth muscle mask its ganglion-stimulating properties in the intact animal and that its ganglionic effects (like those of pilocarpine and unlike those of nicotine) are abolished by atropine.

Sympathomimetic amines.—Epinephrine and closely related substances do not stimulate ganglia. There is general agreement that large amounts of epinephrine and norepinephrine inhibit ganglionic transmission. This effect results from a reduction of the release of ACh and from a decreased sensitivity of the ganglion cell (171). Facilitation by small amounts of epinephrine of ganglionic transmission and of the response of the ganglion to stimulation by nicotine-like substances has been reported by some authors (26, 110, 113, 140, 222) but not by others (136, 144, 145, 146, 213). The discrepancy is probably a result of the methods employed, since most of the positive findings were obtained with perfused or fatigued preparations, whereas intravenous injections into intact animals only caused depression of transmission. Recent observations on the neuromuscular junction sup-

port the view that epinephrine has two different effects: by an action on presynaptic nerve terminals it seems to restore fatigued transmission, whereas it causes neuromuscular block by an action on postsynaptic structures (126). Reserpine depletes the stores of catecholamines present in sympathetic ganglia (160) but does not influence ganglionic transmission (230).

Amphetamine and related substances have a nicotine-like action on the perfused SCG of the cat (110, 185), and depolarization of this ganglion by amphetamine, hordenine, and mescaline has been demonstrated (82). On the isolated heart, however, amphetamine does not seem to have a nicotine-like action, since its effect is not abolished by bretylium, 2,6-xylyl ether of choline (102), or hexamethonium (13).

Ephedrine has been claimed to stimulate isolated atria of the guinea pig by a nicotine-like action (173), but Trendelenburg (229) observed no antagonistic effect of hexamethonium applied in a concentration 100 times higher than that required for abolition of the positive chronotropic response to nicotine.

Miscellaneous substances.—Cardiac glycosides increase the response of the SCG to submaximal preganglionic stimulation and to injections of ACh; larger doses depress ganglionic transmission (115, 116, 177). Stimulation of the ganglion by cardiac glycosides is not abolished by hexamethonium (115). Scillaren A also increases the effect of ACh on the chemoreceptors of the carotid body (36). Veratrum ester alkaloids have similar actions in the perfused SCG of the cat (118). Veratramine has a pure depressing effect on the SCG of the rat, but in this preparation even nicotine, TMA, and ACh fail to elicit stimulation (105, 106).

Intra-arterial injections of small amounts of Substance P facilitate ganglionic transmission as well as the response of the ganglion to injections of ACh; the response to postganglionic stimulation remains unaffected. Larger amounts of Substance P depress ganglionic transmission (14). No stimulation of the quiescent ganglion is observed.

An analysis of the action of "Darmstoff" on the isolated guinea-pig ileum provided evidence for an action of this substance on some nervous mechanism sensitive to morphine and to atropine (233).

Peripheral Ganglion Cells

As already pointed out, many ganglion-stimulating substances are also able to stimulate certain sensory structures. Moreover, it has been shown that chromaffin cells occur in peripheral tissues believed to be innervated only by postganglionic adrenergic nerve endings (30). Under these conditions it is difficult to decide whether a "sympathomimetic" response of an isolated organ to a ganglion-stimulating substance is attributable to a direct effect of this substance on smooth muscle or is mediated through an axon reflex, stimulation of chromaffin cells, or stimulation of intramural ganglion cells. Lack of space does not permit more than a brief reference

to part of the relevant bibliography: isolated bronchial muscle (92), isolated hearts or atria (32, 81, 124, 129, 130, 131, 184, 203, 228), aortic strips (31, 33, 154, 197), perfused rabbit ears (30, 31, 32, 81, 125), isolated nictitating membrane (30, 218). The study of the peristaltic reflex involves mainly cholinergic mechanisms together with sensory structures with multiple possible sites of drug action (15, 16, 17, 27, 28, 122, 132).

Ganglion-Blocking Substances

Hexamethonium and tetraethylammonium.—In most experimental studies as well as in studies of structure-action relationships, hexamethonium or TEA, or both, are used as reference substances. Paton & Perry (170) showed that both agents block ganglionic transmission without causing depolarization and suggested their classification as "competitive" ganglion-blocking substances. These substances are usually assumed to have the same mode of action, but certain differences should be noted: after chronic denervation of the SCG of the cat, hexamethonium fails to block the response to ACh and is itself a stimulant agent; the same phenomenon is produced by the perfusion of normal ganglia with low-potassium solutions; the action of TEA is not changed by these procedures (178). Although hexamethonium and TEA have pure additive effects on the SCG (91), they differ in their effects on the late stages of transmission (stage IV of Rosenblueth) (39). Gill's study of various bis-quaternary compounds (80) showed that the blocking action of methonium compounds (hexamethonium, etc.) depends on the length of the chain; this is not true for the potency of ethonium compounds. Gill concludes that methonium compounds combine with the ACh receptor and an anchoring site, the second attachment preventing the depolarization normally resulting from the combination of the ACh receptor with a trimethylammonium group. Triethylammonium groups, on the other hand, are unable to cause depolarization because of the large substituents; these substances combine less intimately with the receptor (and not with an additional site) and, thereby, prevent the access of ACh to the receptor.

In addition to its ganglionic actions, TEA affects the neuromuscular junction; unlike hexamethonium its effects are not curare-like but resemble those of calcium ions [Stovner (207 to 211)]. An effect of TEA on smooth muscle has also been described (35).

The SCG of the rat (*in situ*) is usually not stimulated by nicotine or ACh (106), but these substances cause stimulation when applied some time after the administration of a moderate amount of hexamethonium. No explanation is yet available for this peculiar phenomenon.

Quaternary ammonium compounds.—The structure-action relationship of ganglion-blocking substances has been discussed by Barlow (10) and by Ing (103). Three series of substances were studied by Fakstorp *et al.* (59, 60, 61), and two others by Hidalgo *et al.* (95) and Horovitz *et al.* (101). Conversion of hexamethonium into the corresponding bis-sulfonium com-

pound results in a loss of potency (11), but substitution of only one of the two quaternary nitrogens by a tertiary sulfonium group leads to compounds with up to 3.3 times the potency of hexamethonium (22). A new group of ganglion-blocking substances has been developed from benzhydryl compounds, some of which have been tried clinically [e.g., N^1-(5-cyano-5-5-diphenylpentyl)-N^1,N^1,N^2-trimethylene-1-ammonium-2-morpholinium bis-methylsulfate, pentacynium bis-methylsulfate] (2, 19, 84, 134, 152).

From several species of magnoliaceae and menispermaceae four alkaloids with curare-like and ganglion-blocking effects have been isolated: menisperine, laurifoline, magnocurarine, magnoflorine (200).

Secondary and tertiary amines.—Mecamylamine (3-methylaminoisocamphane) was the first substance of this group to be studied in detail and to be used clinically (202, 206). Its outstanding property is that it is readily absorbed from the intestine. Its excretion into the urine is inversely related to the pH (155) as is its secretion into the stomach (246); the secretion of mecamylamine into the stomach may be partly responsible for its prolonged action. Inhalation of carbon dioxide (i.e., lowering of the extracellular pH) increases the plasma level of mecamylamine and its hypotensive effect (172). Mecamylamine readily penetrates into the CNS and may cause neurological disturbances (43, 174, 195). When its mode of action was studied in more detail, it was found to differ from that of hexamethonium, and Bennet *et al.* (18) concluded that it does not compete with ACh. Other evidence for a noncompetitive mode of action of mecamylamine was obtained with the frog rectus as test organ (190). The findings of Bennet *et al.* have been confirmed by some (205) and contradicted by others (40).

Since the discovery of mecamylamine, many new secondary and tertiary amines have been studied. The importance of the position of the methyl groups was investigated in a series of six methyl substituted 3-aminonorcamphanes (192). Simplification of the molecule led to a series of polymethylcyclohexylamines (182) and to substituted tertiary hexylamines; as a result of this study "penbutamine" [N,N,2,2,3-pentamethylbutylamine (3)] was found to be equipotent with, and less toxic than mecamylamine (232). Another series of even simpler compounds (hexamethylene-1,6-bis-t-amines) had already been described in 1955 but were found to be less effective than hexamethonium (181).

In contrast to piperidine and propylpiperidine, which are nicotine-like in their action, 2,3- and 2,4-dimethylpiperidine cause ganglion block without initial stimulation (121). Another ganglion-blocking derivative is 1-methyl-3(delta-dimethylaminobutyl)piperidine (163). In 1958 two independent groups of investigators developed and tested 1,2,2,6,6-pentamethylpiperidine (pempidine) (128, 204). Its pharmacology is very similar to that of mecamylamine (40, 90, 156, 205).

Quaternary derivatives of parasympatholytics.—Most of the quaternary derivatives of atropine-like substances are known to have ganglion-blocking properties. Several of these compounds were tested on the SCG of the cat

and compared with hexamethonium and TEA (9). They were found to be potent but the ganglionic block was of short duration; the ratio of their potencies as atropine-like and as ganglion-blocking substances was about 1000:1. Thus, the ganglion-blocking action of quaternary atropine-like substance does not seem to be of therapeutic importance. A different conclusion was reached when some quaternary parasympatholytics were compared with their teritiary parent compounds: quaternization reduced the atropine-like potency by a factor of 10 and somewhat increased the ganglion-blocking potency; the ganglion-blocking action is claimed to be of therapeutic importance (93). 4-Diphenyl-methyl-dl-tropeyl-tropinium bromide has twice the ganglion-blocking potency of hexamethonium and one-fourth of the antimuscarinic activity of atropine; it is claimed to combine both properties when used clinically (161). A study of various phenothiazine, xanthene, and aniline derivatives showed that changes in the molecule close to the ester linkage cause changes in atropine-like activity, whereas changes in the cationic head result in changes in ganglion-blocking activity (193).

Gyermek (85) developed a method which distinguishes between atropine-like and ganglion-blocking agents: pretreatment of mice with carbachol does not interfere with the mydriasis produced by atropine but prevents that produced by ganglion-blocking substances. Quantitative determination of ganglion block caused by quaternary parasympatholytics is impossible, but an indication of the predominance of peripheral or ganglionic action may be obtained.

Miscellaneous substances.—Paraldehyde, methylpentynol, and methylpentynol carbamate block ganglionic transmission, the latter two by depressing the output of ACh as well as by reducing the sensitivity of the ganglion cells (142, 183). Chlorpromazine and other phenothiazine derivatives depress synaptic transmission when injected into the perfused SCG or applied topically; after intravenous injections of these substances into whole animals, their adrenolytic effects are usually so strong that any ganglion-blocking action is masked (25, 109, 198, 239). On the neuromuscular junction chlorpromazine is curare-like in some respects, but it also reduces the liberation of ACh (212).

Compound 48/40, injected into the perfused SCG, blocks ganglionic transmission without interfering with the release of ACh (73). Monoamine oxidase inhibitors of different chemistry (iproniazide, harmine, and *p*-tolyl ether of choline) cause a very slowly developing block of ganglionic transmission (74).

A new group of antinicotine substances has been described by Surber (214); they antagonize the effects of nicotine without depressing ganglionic transmission or the response to other "nicotine-like" substances, such as dimethylphenylpiperazine. Their mode of action is not yet known.

Release of acetylcholine from preganglionic nerve endings.—Ganglionic block is usually defined as failure of synaptic transmission. Reduction or abolition of the output of ACh in response to preganglionic nerve stimula-

tion is one of the situations resulting in block of synaptic transmission. This is experimentally verifiable, but it should be remembered that the experimental demonstration of an abolition of the release of ACh gives only little indication of the mechanisms involved. Impairment of conduction of the nerve impulse, interference with the synthesis, with the storage, or with the release mechanism of ACh may all result in a decreased liberation of this substance. Detailed studies of these mechanisms are rare, and the problem is further complicated by the fact that many substances that are able to affect the release of ACh also have some effect on postsynaptic structures (sympathomimetics, local anesthetics, anesthetics, etc.).

Hemicholinium has a weak ganglion-blocking action, when tested on the SCG of the cat (240). Experiments on motor nerves and skeletal muscle indicate that its site of action is probably the presynaptic nerve ending (135). When perfused through the SCG, hemicholinium reduces the synthesis of ACh, an action which is antagonized by the addition of choline to the perfusate (137). Studies with various brain tissues indicate that hemicholinium does not affect the cholineacetylase system but interferes with the transport of choline into the cell and into mitochondria (71, 137). For a discussion of the importance of choline for the replenishment of the ACh stores see (53, 175).

Various ions are known to affect the release of acetylcholine in response to preganglionic stimulation; this aspect has been reviewed by Birks & MacIntosh (20). The effect of lead ion resembles that of magnesium ion: the liberation of ACh is reduced, the response of the ganglion to injected ACh remains normal, and an increase in the calcium ion concentration restores ganglionic transmission (123).

Some mechanisms involved in ganglionic blockade.—The problem whether central effects of ganglion-blocking substances contribute to their hypotensive action is the subject of contradictory evidence. Several groups of workers injected ganglion-blocking substances into the blood supply of the head which was perfused from a donor dog; Heymans *et al.* (94) and Murray *et al.* (159) observed no fall in systemic blood pressure of the recipient dog, whereas Lape & Hoppe (127) reported positive results. Under these conditions, negative results are of greater weight, since a leak from the circulation of the head to the systemic circulation would result in positive findings. Hence supra-spinal centers do not seem to be involved in the hypotensive response to ganglion-blocking substances, but these experiments do not exclude the spinal cord as a possible site of action. The electrical recording of preganglionic activity provides another approach to this problem; the results are equally controversial: Dontas & Nickerson (48) observed a pronounced reduction of activity after hexamethonium and TEA, whereas McCubbin & Page (150) found the activity to be increased after hypotensive doses of TEA or mecamylamine. The latter also observed an unchanged preganglionic response to occlusion of the carotid arteries, while postganglionic fibers were silent.

Gallagher (70) demonstrated that the systemic injection of hexamethonium completely abolishes the sympathetic tone of a perfused hindleg with intact nervous connections. But there have been a number of reports of autonomic pathways resistent to ganglionic blockade. Large doses of ganglion-blocking substances fail to prevent pressor responses to supramaximal stimulation of the splanchnic nerve (149). In both rats and dogs the expected hypotensive response to TEA and to clorisondamine is obtained only under anesthesia and not in unanesthetized animals (148, 166); it has been postulated that anesthesia may interfere with compensatory mechanisms which are not blocked by ganglion-blocking substances. These hypothetical compensatory mechanisms seem to be species dependent, since unanesthetized monkeys and rabbits respond to chlorisondamine (148). The existence of pathways that are not affected by ganglionic blockade may be involved in the phenomenon described by Giertz *et al.* (79): the pressor response to certain analeptic substances (pentylenetetrazol, nikethamide) is believed to be of central origin and is antagonized by azamethonium, but there is a mutual antagonism, i.e., larger amounts of the analeptics can overcome the effect of the ganglion blocker. The characteristics of this mutual antagonism vary with species and with the type of anesthesia employed. An alternative explanation for this phenomenon would be the assumption that the analeptics have, in addition to their central effects, a direct action on ganglion cells.

The phenomenon of tolerance has also been connected with the existence of pathways not blocked by otherwise fully effective doses of ganglion-blocking substances. Postganglionic electrical recording of the activity of renal sympathetic nerves revealed activity persisting after ganglion block; the reflex increase of activity during occlusion of the carotid arteries also persisted (167). Various other mechanisms may contribute to tolerance to ganglion-blocking substances. The "potentiation" of the pressor response to various substances during ganglionic blockade is a well-known phenomenon. Blockade of the compensatory reflexes alone does not seem to be the only factor involved. About equieffective doses of eight different pressor substances were injected before and after pentolinium; the degree of "potentiation" varied from 5 to 240 per cent (89). A much more uniform increase of the response should be expected, if pressoreceptors were of major importance. The authors, therefore, suggest an effect of pentolinium on intrinsically liberated ACh [see (7)]. Furthermore, bilateral removal of the carotid sinus region, together with bilateral vagotomy, does not prevent the increase of pressor responses by ganglion-blocking substances (139). During infusions of ganglion-blocking substances, it has been found that the carotid occlusion reflex is abolished before maximal "potentiation" develops, and during prolonged infusions the response to occlusion of the carotid arteries appears again, although the "potentiation" of pressor responses persists or even increases (167).

There is thus growing evidence for the view that blockade of com-

pensatory mechanism alone cannot explain the phenomenon of "potentiation" of pressor responses by ganglion-blocking substances. An increased responsiveness of smooth muscle during ganglionic blockade has been demonstrated by Mantegazza *et al.* (141): the response of the nicitating membrane to postganglionic stimulation as well as to injections of norepinephrine is increased by hexamethonium and pentolinium. Administration of chlorisondamine for three weeks causes pronounced supersensitivity of the salivary glands and of the nictitating membrane of the cat (52). This latter type of supersensitivity may be quite different from that observed in acute experiments; it may be a result of prolonged "inactivity" of the tissues involved rather than to an action on them of the ganglion-blocking substances.

Parasympathetic ganglion cells do not seem to differ from sympathetic ones in their reaction to ganglion-blocking agents. Comparisons have been made either by simultaneous recording of action potentials from postganglionic fibers of the SCG and of the ciliary ganglion of the cat (64) or by recording the response of the heart to alternating stimulation of the vagus and of the pre- and postganglionic sympathetic fibers (180).

Paton & Perry (170) classified those ganglion-blocking substances that do not interfere with the release of ACh from presynaptic nerve endings as "depolarizing" or as "competitive" agents according to their ability or inability to depolarize ganglion cells. The depolarizing action of some of the former agents (TMA) fully accounts for their ganglion-blocking action, whereas the blocking action of nicotine is of considerably longer duration than its depolarizing effect; in analogy to similar phenomena observed at the neuromuscular junction, the second phase of the blocking action of nicotine is thought to be due to "competitive" blockade. This hypothesis is in agreement with the observation that ganglion cells do not respond to potassium ion during the first phase of the nicotine block, but are excitable by potassium ion during the second (225), just as they respond to this ion during blockade by typical "competitive" blocking substances such as TEA (1) or "curarine" (23). The classification as "depolarizing" and "competitive" blocking agents may need some modification, since chlorisondamine and mecamylamine, which do not cause initial stimulation of ganglion cells (and, therefore, presumably do not cause depolarization), have been found to be noncompetitive (or unsurmountable) antagonists of ACh when tested on the frog rectus (190).

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NEUROMUSCULAR PHARMACOLOGY[1,2]

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The acetylcholine released from motor-nerve endings is believed to become adsorbed to receptor substances of the muscle end-plate, following which the permeability of the end-plate for Na^+ and K^+ increases temporarily, the end-plate becomes depolarized, and the end-plate and muscle action potentials and muscle fiber contraction are successively initiated (1, 2). Disturbance of neuromuscular transmission usually results from either deficient or excessive action of acetylcholine on the motor end-plate, or from the action of drugs, changes in ionic concentration, or disease which simulates these effects. Neuromuscular block may occur without any change in the resting potential of the muscle membrane (nondepolarizing block), or following reduction in the resting potential (depolarizing block). It may be characterized descriptively in terms of whether or not it inhibits the depolarizing action of acetylcholine and is reversible by acetylcholine.

NONDEPOLARIZING BLOCKING AGENTS

Neuromuscular block without alteration in resting membrane potential may result from deficient release of acetylcholine from the motor-nerve endings or from inhibition of the depolarizing action of acetylcholine on the motor endplates.

Agents that Inhibit Release of Acetylcholine

Toxins.—The paralysis in botulism (3) and in puffer poisoning (4) is a result of prevention by these toxins of release of acetylcholine from terminal nerve endings. The muscle remains responsive to direct stimulation or to exogenous acetylcholine, but becomes unresponsive to stimulation through the nerve after 30 minutes exposure to the toxin (5). Release of acetylcholine from parasympathetic postganglionic nerve endings is also prevented. The motor paralysis produced by the toxin secreted by salivary glands of the Rocky Mountain wood tick, *Dermacentor andersoni,* has similarly been attributed to deficient release of acetylcholine from nerve endings (6). However, the progression of the ascending paralysis resembles that of

[1] The survey of the literature pertaining to this review was concluded in July, 1960.

[2] Abbreviations used in this chapter include: DFP (diisopropyl phosphorofluoridate); DAM (diacetyl monoxime); EA1814, TMB-4 [1,1′-trimethylene-bis-(4-formylpyridinium bromide) dioxime]; EPN [O-ethyl O-(4-nitrophenyl) phenylphosphonothioate]; MINA (monoisonitroso acetone); OMPA (octamethyl pyrophosphortetramide); 2-PAM (pyridine-2-aldoxime methiodide); P2S (pyridine-2-aldoxime methanesulfonate); TEPP (tetraethyl pyrophosphate).

polyneuritis more than botulism (7). This suggests that the tick toxin may block conduction in motor-nerve fibers (8), whereas botulinus toxin more directly impairs release of acetylcholine from the nerve endings. The paralysis produced by diptheria toxin is even more clearly the result of impaired conduction in the motor nerve, as transmission at the neuromuscular junction is not affected. There is some evidence that this toxin may diminish synthesis of acetylcholine within the neurone (9).

Hemicholinium (HC-3).—This compound, which inhibits the synthesis of acetylcholine, blocks transmission at the neuromuscular junction and at the endings of postganglionic parasympathetic nerves. These actions are antagonized by choline, and, to a lesser extent, by analogues and esters of choline (10, 11). Diphenylbutylacetic acid appears to have an effect similar to that of hemicholinium in smooth muscle (12).

Compounds that Inhibit Depolarizing Action of Acetylcholine

Inhibition of the depolarizing action of acetylcholine on the motor end-plate ("acetylcholine-inhibitory" block) occurs in at least three situations:

Block which occurs without change in the resting potential of the muscle membrane, and which is reversible by acetylcholine.—The block produced by *d*-tubocurarine is of this type. Because this drug exerts its effect by competing with acetylcholine for receptor sites at the end-plate, the resulting block has been termed "competitive" (13). This type of block is characterized by inhibition of the depolarizing action of injected acetylcholine and by reversal of the block following the injection of sufficient acetylcholine or anticholinestrease compound. Attempts have been made to identify the receptor substances of the end-plate by their combination with C^{14}-labelled nondepolarizing blocking agents such as *d*-tubocurarine, dimethyl-*d*-tubocurarine, the triethiodide of gallamine (TRIEG), and dimethylisochondodendrine, which complete with acetylcholine for the receptor substances. The substances in eel electric tissue and mammalian skeletal muscle with which these compounds combine have been identified as acid mucopolysaccharides by Chagas (14) and as proteins by Ehrenpreis (15).

Numerous derivatives of *d*-tubocurarine, and related alkaloids, continue to be introduced as muscle-relaxant aids to anesthesia (16). Most are long acting, and apnea caused by excessive administration or unusual reactivity frequently occurs, even though the block can usually be reversed by anticholinesterase compounds, particularly neostigmine (Prostigmin) and its analogues (17). This reversal can sometimes be aided by the administration of potassium salts, epinephrine, or ephedrine (18). Occasionally, however, for reasons that are not clear, irreversible curarization occurs, particularly in elderly, debilitated patients with electrolyte disturbance (19, 20).

The antibiotic, neomycin, has been found to produce neuromuscular block of the nondepolarizing type when administered parenterally in large doses (21, 22). The block is potentiated by ether and antagonized by neostigmine. Streptomycin has a similar but much less marked effect (23).

Block not associated with any change in membrane potential, but not reversible by acetylcholine.—This type of block occurs several minutes after the exposure of frog muscle to acetylcholine or other depolarizing compounds and persists even after recovery of the muscle membrane from the initial depolarization. It is attributable to the development of refractoriness of the motor end-plate to acetylcholine (24). Gamma-aminobutyric acid, glutamine, and some other amino acids inhibit transmission at the crustacean neuromuscular junction and in certain mammalian central synapses (25, 26). The mechanism of these blocks is not known. They are not reversed by acetylcholine, but the gamma-aminobutyric acid block is reversed by picrotoxin. Gamma-aminobutyric acid is present in relatively high concentration in the brain and may be a physiological inhibitor of transmission (27).

Block associated with hyperpolarization or stabilization of the muscle membrane.—The weakness of hypokalemia is probably attributable, in part, to hyperpolarization or stabilization resulting from an increase in the ratio of intracellular to extracellular potassium concentrations (28). The neuromuscular block produced by tetrodotoxin, obtained from the goldfish, has been attributed to stabilization of the muscle membrane by inactivation of the sodium-carrying mechanism (29).

DEPOLARIZING BLOCKING AGENTS

When the end-plate region is in the depolarized state it is inexcitable. This type of block is produced by an excessive concentration of acetylcholine, by anticholinesterase compounds, by choline, and in most animal species by decamethonium and succinylcholine. During the initial stages of neuromuscular block produced by these compounds, there is abnormally prolonged depolarization of the muscle membrane, most marked in the region of the end-plate, and no inhibition of the depolarizing action of injected acetylcholine or anticholinesterase compound; in fact, these compounds intensify the block by their additive effect. However, the neuromuscular block persists after return of normal membrane polarity, because the end-plate develops refractoriness to acetylcholine (30). The properties of this "desensitization" block, which has been ascribed to alteration of the receptor substances from an "effective" to a "refractory" state, vary to some extent with different depolarizing agents.

Anticholinesterase Compounds

Organophosphorus compounds.—New agents have been employed as insecticides, chemical warfare agents, and pharmaceutical agents. Most are of high potency, and may be absorbed through any body surface. Parathion, malathion, O-ethyl-O-(4-nitrophenyl) phenylphosphonothioate (EPN), phosdrin, mipafox, tetraethyl pyrophosphate (TEPP), and octamethyl pyrophosphortetramide (OMPA) (Table I) have been widely used as agricultural insecticides, and their indiscriminate dispersal has resulted in a number

of instances of poisoning, some of them fatal (31, 32). Until recently parathion was the most widely used of the organophosphorus insecticides, and most intoxications, including 6000 in Japan alone, have been caused by this compound. In recent years, there has been increasing use of malathion, which, although less toxic than parathion to mammals, has also produced poisoning after ingestion (33, 34). The organophosphorus chemical warfare agents have been termed nerve gases because of their effect on the central nervous system. Sarin and tabun are among the more important of these, and several instances of intoxication have occurred following exposure to these compounds (35). Diluted solutions of diisopropyl phosphorofluoridate

TABLE I

ORGANOPHOSPHORUS ANTICHOLINESTERASE AGENTS THAT HAVE PRODUCED INTOXICATION IN MAN

General formula:

Symbol or Common Name	Chemical Name
A. Compounds where X = halogen or CN	
Sarin (GB)	Isopropyl methylphosphonofluoridate
DFP	Diisopropyl phosphorofluoridate
Tabun (GA)	Ethyl-N-dimethyl phosphoramidocyanidate
Mipafox (Isopestox)	N,N′-Diisopropylphosphorodiamidic fluoride
B. Compounds where X = alkyl, alkoxy, or aryloxy	
Phosdrin	Dimethyl 1-methyl-2-carbomethoxyvinyl phosphate
Paraoxon	Diethyl 4-nitrophenyl phosphate
C. Thiol- and thionophosphorus compounds	
EPN	O-Ethyl-O-(4-nitrophenyl) phenylphosphonothioate
Parathion	O,O-Diethyl-O-(4-nitrophenyl)phosphorethioate
Malathion	O,O-Dimethyl S-(1,2-dicarbethoxyethyl) phosphorodithioate
D. Derivatives of pyrophosphorus acid	
TEPP	Tetraethyl pyrophosphate
OMPA	Octamethyl pyrophosphortetramide
E. Compound containing a quaternary nitrogen	
Echothiophate (phospholine)	O,O-Diethyl-S-2-trimethylammonium-ethyl phosphonothiolate iodide

(DFP), TEPP, and echothiophate (Phospholine) have been used locally in the management of glaucoma (36). Dilute solutions of DFP, TEPP, hexaethyl tetraphosphate (HETP), OMPA, sarin, and echothiophate have been administered orally or intramuscularly in clinical trials in the management of myasthenia gravis (37).

Quaternary ammonium compounds.—These have been used mainly in the management of myasthenia gravis and, to a lesser extent, of glaucoma and paralytic ileus. Drugs used in the diagnosis and management of myasthenia gravis include neostigmine, pyridostigmine (Mestinon), edrophonium (Tensilon), and ambenonium (Mytelase) (37). Clinical trials have also been carried out with bis-neostigmine (BC-40), bis-pyridostigmine (hexamarium, BC-51, (38a), galanthamine, and lycoramine (38b). Demecarium bromide (BC-48) has recently been used in the management of glaucoma (39).

Anticholinesterase activity.—The inhibitory activity of sarin against the cholinesterase enzymes of the red cells, muscle, and brain is greater than that of the other anticholinesterase compounds, being approximately five times that of tabun, 10 times that of TEPP, 100 times that of DFP or neostigmine, and 4000 times that of parathion (40). Sarin, tabun, and DFP combine with cholinesterase *in vitro* almost irreversibly during the first hour of their reaction and, hence, produce the most prolonged and cumulative effects. The combination of TEPP or parathion with cholinesterase is slightly reversible, whereas the combination of neostigmine with the enzyme is entirely reversible.

Manifestations of anticholinesterase intoxication in man.—All anticholinesterase compounds produce muscarine-like and nicotine-like effects. Organophosphorus compounds of high lipoid solubility, such as sarin, parathion, DFP, malathion, EPN, phosdrin, and tabun, also produce marked central neural effects, while those of low lipoid solubility, such as TEPP, OMPA, and echothiophate (41, 42), and quarternary ammonium compounds, have less effect on the central nervous system. The muscarine-like effects include miosis, increased salivary and bronchial secretion, bronchoconstriction, sweating, and gastrointestinal symptoms, and the central neural effects may include anxiety, headache, convulsions, coma, and respiratory depression (37, 40, 43). The nicotine-like effects on skeletal muscle begin with increased fatiguability and mild generalized weakness which is increased by exertion. This is followed by involuntary muscular twitching, fasciculations, and sometimes cramps. If the exposure has been sufficiently marked, there occurs severe weakness, which is generalized and includes the muscles of respiration. A decrease in tidal volume and respiratory rate and an increase in airway resistance generally occur. Weakness of the muscles of the tongue and pharynx may aggravate upper airway obstruction. The relative importance of central depression of respiration, peripheral neuromuscular block, and bronchoconstriction in causing death varies in different species and with different anticholinesterase compounds and routes of ad-

ministration (44). In man, it is likely that failure of the central drive of respiration is the most important factor, with peripheral neuromuscular block next in importance. Bronchoconstriction appears to be less important in man than in most experimental animals, although airway obstruction resulting from inspissated secretions may occur and may seriously impair ventilatory exchange.

The neuromuscular block produced by anticholinesterase compounds is mainly attributable to the local accumulation of acetylcholine resulting from inhibition of muscle cholinesterase. There occurs an increase in amplitude and frequency of spontaneously occurring miniature end-plate potentials, resulting in the spontaneous appearance of propagated action potentials which cause twitches in individual muscle fibers (fibrillations) and in groups of fibers (fasciculations). When a nerve impulse reaches the myoneural junction, there is increased amplitude and striking prolongation of the end-plate potential, and repetitive firing may take place (45). The muscle-action potential response to a single nerve stimulus is only slightly affected (46), but the responses to subsequent stimuli of a train are progressively reduced, and muscle contraction is not sustained (47). Following neuromuscular block produced by TEPP or DFP, there is recovery from the block before there has been measurable restoration in cholinesterase activity, suggesting that either junctional transmission is not related solely to cholinesterase activity, or is influenced by recovery that is within the limits of experimental error. Some anticholinesterase compounds appear to affect neuromuscular transmission by mechanisms in addition to cholinesterase inhibition. Very large doses of DFP produce further reduction in the muscle response to a single nerve stimulus even after complete inactivation of cholinesterase. Furthermore, this block is accentuated by *d*-tubocurarine, which antagonizes more moderate degrees of anticholinesterase neuromuscular block (47).

Residual effects.—A few instances of persistent paralysis of the extremities with muscle atrophy and loss of tendon reflexes have occurred following exposure to parathion, mipafox, malathion, and EPN (48). Electromyographic studies have shown no evidence of neuromuscular block but have revealed changes similar to those seen in peripheral neuritis. The disorder resembles the peripheral neuritis and demyelinization that occurs more frequently after acute or chronic exposure to triorthocresylphosphate (Ginger Jake paralysis) (49, 50). Demyelinization has been observed in the peripheral nerve and spinal cords in experimental animals following the administration of mipafox, DFP, and other dialkyfluoridates, or triorthocresylphosphate (51). Alkyl organophosphorus compounds containing fluorine appear to be the most neurotoxic, perhaps as a result of liberation of ionic fluorine following rupture of the P-F bond by cholinesterase in the nervous system (52).

Treatment of Anticholinesterase Intoxication

This treatment consists of removal of the toxic agent, administration of

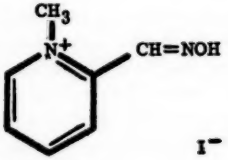
atropine and oxime, removal of oral secretions, maintenance of a patent airway, and mouth-to-mouth artificial respiration if needed (35, 53).

The administration of large doses of atropine ameliorates the muscarine-like effects of anticholinesterase compounds, and to a lesser extent the central neural effects, but has no influence on weakness resulting from neuromuscular block. Until recently, there has been no clinically useful means of accelerating recovery from the neuromuscular block. However, during the past several years, Wilson and others have demonstrated that cholinesterase inhibited by organophosphorus anticholinesterase compounds may be reactivated *in vitro* by derivatives of hydroxamine acid [R − C(= O) − NHOH], and to a greater extent by a number of oximes [R − C(= NOH) − R] (54). Both groups of compounds also react with these inhibitors to inactivate them directly. The action of organophosphorus anticholinesterase agents on smooth, cardiac, and skeletal muscle of experimental animals can be reversed by hydroxamic acid derivatives (54) and oximes (55) and their lethal effects reduced (56), although species differences are wide.

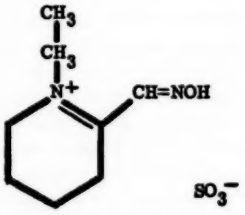
Protection and reactivation by oximes of cholinesterase enzymes inhibited by anticholinesterase compounds in vitro.—The oximes pyridine-2-aldoxime methiodide (2-PAM), pyridine-2-aldoxime methane sulfonate (P2S), 1,1′-trimethylene-bis-(4-formylpyridinium bromide) dioxime (EA 1814, TMB-4), diacetyl monoxime (DAM), and monoisonitroso acetone (MINA) (Fig. 1) cause moderate protection and reactivation of cholinesterase enzymes inhibited by organophosphorus compounds such as sarin (57 to 62). Reactivation by oxime is less after prolonged contact of organophosphorus inhibitor with enzyme. 2-PAM, TMB-4, and to a lesser extent DAM, cause slight protection and very slight reactivation of human cholinesterase enzymes inhibited by quaternary ammonium compounds such as neostigmine and pyridostigmine, but not ambenomium (62) or physostigmine (63). The inhibition of cholinesterase by organophosphorus compounds has been considered to occur by direct phosphorylation of some group at the active center of the enzyme, and reversal of this inhibition by oximes to be caused by displacement of the enzymic group from the phosphorus atom (57). Since the quaternary ammonium anticholinesterase compounds do not contain a phosphorus atom, it is evident that the oximes may reverse cholinesterase inhibition by a more general mechanism than displacement of phosphorus (62).

Reversal by oximes of neuromuscular block produced by anticholinesterase compounds.—The neuromuscular block produced in man by the intraarterial injection of organophosphorus anticholinesterase compounds such as sarin, or quaternary ammonium compounds such as neostigmine, bis-neostigmine, pyridostigmine, bis-pyridostigmine, or ambenomium, is promptly and strikingly reversed in the injected extremity immediately after the intraarterial injection of 2-PAM, DAM, or TMB-4 (62). The effect of each of these oximes on neuromuscular block produced by organophosphorus and quaternary ammonium anticholinesterase compounds is striking and equal, in contrast to lesser ability and marked differences in protection and reactiva-

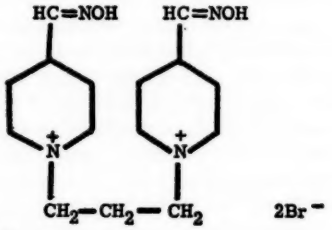
Pyridine aldoxime methiodide (2-PAM)



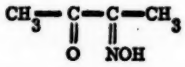
Pyridine aldoxime methane sulfonate (P2S)



1,1'-Trimethylene-bis-(4-formylpyridinium bromide) dioxime (EA 1814, TMB-4)



Diacetyl monoxime (DAM)



Monoisonitroso acetone (MINA)

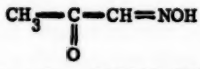


FIG. 1. Formulae of the oximes 2-PAM, P2S, TMB-4, DAM, and MINA.

tion of cholinesterase, including that of muscle, *in vitro*. This suggests that either the enzyme in intact muscle may be particularly accessible to the action of the oximes, or the oximes may restore or protect neuromuscular transmission by another mechanism than restoration or protection of muscle cholinesterase. High concentrations of 2-PAM have been found to produce a reversible block of the action of acetylcholine on isolated frog muscle (64),

while lower concentrations of 2-PAM potentiate the action of acetylcholine, perhaps as a result of cholinesterase inhibition. It seems likely that the reversal by oximes of anticholinesterase neuromuscular block is not only attributable to the reversal of cholinesterase, inhibition, but also, and perhaps mainly, to inhibition of the action of acetylcholine.

The intraarterial injection of high concentrations of 2-PAM or TMB-4 produces a transient neuromuscular block, whereas DAM has no effect (62). The block does not alter the affect of acetylcholine on neuromuscular transmission and is not reversed by acetylcholine or neostigmine, but it is increased by the prior administration of neostigmine. The block may be attributable to a direct action on the muscle fiber (65).

The intravenous administration to man of 500 to 2000 mg. of 2-PAM or DAM, or 150 to 250 mg. of TMB-4, ameliorates to a moderate degree generalized weakness resulting from any of these anticholinesterase compounds (62, 66, 67). Muscular fasciculations are usually reduced to a lesser degree, but may be unchanged. Muscarine-like and central neural symptoms produced by sarin or quaternary ammonium anticholinesterase compounds are not appreciably influenced by oxime administration (62), but those produced by parathion have been reported to be ameliorated (66, 68). It is advisable to administer large doses of atropine intravenously, in addition to oxime, in the management of serious anticholinesterase intoxication.

Distribution and effects of oximes.—The intravenous administration of 1000 mg. (15 mg./kg.) of 2-PAM or DAM produces a plasma concentration of approximately 2 mg./100 ml. (69). 2-PAM is removed primarily by renal excretion and has a half life of 0.9 hour. The renal clearance of 2-PAM is almost three times that of creatinine, with 80 per cent of injected material appearing in the urine as an altered derivative within six hours and most within the first half hour. In contrast, DAM is removed more slowly, primarily by means other than renal excretion and has a half life of 7.2 hours. The renal clearance of DAM is only 6 per cent that of creatinine, and less than 10 per cent of injected material appears in the urine in six hours. Observations in experimental animals indicate that the liver is the main site of destruction of DAM (70). Both 2-PAM and DAM appear to be widely dispersed in total body water, except that 2-PAM, like most quaternary ammonium compounds, does not enter the spinal fluid. DAM, a tertiary amine, does enter the spinal fluid. Rapid intravenous injection of 2-PAM may result in transient mild weakness, diplopia, blurred vision, dizziness, impairment of accommodation, and occasionally headache, nausea, and tachycardia (69). DAM may produce a burning sensation at the site of injection radiating up the injected vein, followed by moderate giddiness, drowsiness, a sensation of warmth and tingling in the abdomen and chest, tachycardia, slight increase or decrease in blood pressure, and mild postural hypotension (62). Rapid injection of large doses may cause bitter taste, paresthesias and decreased position sense in the extremities, decreased sweating, transient loss of consciousness, clonic movements of

the head, and decreased amplitude of the electroencephalogram and of the T-wave of the electrocardiogram (69).

Depolarizing Muscle Relaxants

Biphasic neuromuscular block.—The characteristics of the neuromuscular block produced by most depolarizing agents, including choline, succinylcholine, and decamethonium, depend on the degree and duration of the block. In most instances, signs of stimulation, such as muscular fasciculations occur first. This is followed by the usual manifestations of depolarizing block: equal depression of the muscle-action potentials evoked by successive nerve stimuli, no inhibition of the depolarizing action of acetylcholine, and enhancement of the block by acetylcholine or anticholinesterase compounds. When the block is of severe degree or prolonged duration, these characteristics may change to those of the nondepolarizing, competitive type: progressive depression of successive evoked potentials, inhibition of the depolarizing action of acetylcholine, and reversal of the block by acetylcholine or anticholinesterase compounds (47, 71, 72). In addition, there may be gradually decreasing reactivity (tachyphylaxis) to the effect of repeated doses of depolarizing relaxant (73). The characteristics of this biphasic block have been studied on isolated human intercostal nerve-muscle preparation (74, 75, 76). The second phase of the block may be similar in mechanism to the "desensitization" block produced by acetylcholine, although the latter is not reversible by acetylcholine or anticholinesterase compounds (24, 77). In both instances prolonged or intensive exposure of the end-plate receptors to the depolarizing agent appears to result in altered sensitivity of these receptors to the physiologic transmitter. There is some evidence that the excitation mechanism of the entire muscle membrane may be interfered with, and that the membrane-contractile link may be blocked (78).

Succinylcholine.—This compound has relatively short duration of action and is widely used for brief muscular relaxation, or, in fractional doses or infusion, for prolonged relaxation (79). It is hydrolyzed by plasma cholinesterase to choline and succinylmonocholine, which is in turn hydrolyzed by plasma cholinesterase and by an enzyme in the liver to choline and succinic acid. The hydrolysis of succinylcholine is delayed and its neuromuscular blocking action prolonged when plasma cholinesterase activity is reduced (80). This may occur as a result of hepatic insufficiency or prior administration of an anticholinesterase compound, or, rarely, in otherwise normal individuals as an inherited familial trait. Qualitative differences in plasma cholinesterase may also result in delayed hydrolysis of the drug (81, 82, 83). A selective inhibitor of plasma cholinesterase, hexafluorenium, has been employed to increase the duration and intensity of muscle-relaxing effect of succinylcholine in both anesthetized and unanesthetized subjects (84). The action of the latter drug may be slightly shortened by the intravenous

administration of concentrated human plasma cholinesterase (Cholase) (85), but not enough to be of practical value. Unusual reactivity to the neuromuscular blocking action of succinylcholine may result not only from reduced plasma cholinesterase activity, but also from dehydration, hypokalemia, and possibly hyponatremia, hypocalcemia, and hypermagnesemia (81). It may also occur without apparent cause, perhaps as a result of abnormal fixation of the drug to the end-plate receptors (80, 86). Careful administration of edrophonium or neostigmine sometimes reverses intense succinylcholine block (72), and occasionally administration of potassium chloride may reverse the block, or make it reversible by anticholinesterase compounds (87). This action of potassium is similar to its effect on the nondepolarizing block caused by *d*-tubocurarine, and it is possible that it may occur when the succinylcholine block has become of the same type. Or, it may be due to the replacement of potassium ions which are lost from muscle under the influence of depolarizing agents, as reflected by increased plasma concentration and urinary excretion of this ion (88).

The distribution, metabolism, and elimination of muscle relaxants has been reviewed by Kalow (89), and fetal transmission by Baker (90). Unlike most other drugs with molecular weight of less than 1000, succinylcholine, decamethonium, and *d*-tubocurarine do not cross the placental barrier in significant amount (90, 91). There is some evidence that the fetus may have decreased reactivity to depolarizing agents and increased reactivity to nondepolarizing agents (90).

The combined use of depolarizing and nondepolarizing relaxants presents increased problems (92) and is generally not advisable, even though it decreases postanesthetic muscle pain attributable to the initial stimulating effect of the former (93). Succinylcholine may be used to induce endotracheal intubation and may then be followed by a nondepolarizing drug for maintenance of relaxation if the latter is delayed until the effect of the former has worn off (92). It has been suggested that caution be exercised in the use of procaine in patients receiving succinylcholine, since procaine inhibits plasma cholinesterase (85). Procaine may also affect neuromuscular transmission by inhibiting the release of acetylcholine from motor-nerve endings, by competing with it for end-plate receptors, and by a stabilizing effect on the postjunctional membrane. Oxytocin has also been found to prolong the action of succinylcholine, but the mechanism is not known (94). There is some evidence that ganglion-blocking agents may intensify the neuromuscular effects of both depolarizing and nondepolarizing relaxants (81).

Newer depolarizing blocking agents.—These include hexamethylene-1, 6-biscarbaminoyline bromide (Imbretil), a new muscle relaxant (95), a series of lipoid-soluble long chain derivatives of tetramethylammonium (96), a group of benzyltrimethylammonium iodides (97), a series of bis-onium compounds derived from substituted tropane nuclei (98), and a family of plant pyrrolizidine alkaloids (99).

Tissue extracts.—Gamma-butyrobetaine, which has been extracted from muscle and thymic tissue (100), and other quaternary ammonium compounds which have been extracted from thymic tissue (101), are capable of producing neuromuscular block of the depolarizing type in experimental animals. Maltoxin, an amine from brew malt rootlet, exerts a similar effect (102).

OTHER CHEMICAL AND PHYSICAL AGENTS

Alterations in Electrolyte Concentration

Alterations in concentration of potassium, calcium, sodium, or magnesium in the extracellular fluid may result in derangement of excitation, not only of the motor end-plate, but also of the muscle fiber, nerve, and cardiac and smooth muscle.

Potassium.—The normal resting potential of the muscle membrane is attributed mainly to the concentration gradients of potassium and chloride ions across the membrane; in experimental animals the resting potential varies with these gradients (103). Excitation of muscle is associated with entry of sodium ions and a somewhat smaller loss of potassium ions (104). In normal subjects, rest or administration of glucose, epinephrine, or potassium chloride causes movement of potassium into muscle from the extracellular fluid (28, 105). Exercise, administration of large doses of insulin, deficient intake of potassium, excessive loss of potassium in vomitus, stool, or urine, or alkalosis causes movement of potassium out of muscle. The effect of insulin *in vivo* is probably caused by net movement of potassium into the liver, since, *in vitro*, insulin causes potassium to move into muscle. The latter has been attributed to hyperpolarization of the muscle membrane by the insulin (106).

In patients with periodic paralysis, rest, glucose, insulin, or epinephrine causes an exaggerated movement of potassium into muscle (28). Induced by the prior ingestion of carbohydrate and by rest, the initial event during an attack of periodic paralysis is an abnormal uptake of potassium by muscle. This is followed by a decrease in reactivity of the muscle to the depolarizing action of acetylcholine. This finding is suggestive of an abnormal increase in muscle-membrane potential (hyperpolarization of the muscle membrane) attributable to an increase in the concentration gradient of potassium, but confirmation awaits direct measurement of membrane potential. There is a decrease in muscle responsiveness to nerve stimulation, in propagation of excitation and in contractility, resulting in paralysis. Following the administration of potassium chloride, there is first a return of the extracellular concentration of potassium toward normal. This results in a return toward normal of reactivity to acetylcholine and probably of the muscle membrane potential, which results from a return toward normal of the concentration gradient of potassium. There is a partial return of muscle responsiveness, propagation of excitation, and contractility which results in partial return of strength and increased muscle activity. The latter causes an exaggerated

extrusion of potassium from muscle, which is in turn followed by accelerated improvement in muscle function and strength. Conn (107) has reported that attacks of periodic paralysis are preceded by increased urinary excretion of aldosterone, decreased excretion of sodium, and movement of sodium into muscle. However, these changes do not occur in all patients, and their importance is not clear.

Movement of potassium out of cells and into the extracellular fluid may take place as a result of intravascular hemolysis, tissue damage as in crush injury, or acute acidosis; hyperkalemia may follow, particularly if there is also reduced renal blood flow or other cause of renal insufficiency. Movement of potassium out of muscle into the extracellular fluid also occurs in a rare heritable disease, adynamia episodica hereditaria, described by Gamstorp (108). This is in many ways the opposite of familial periodic paralysis. Attacks of weakness occur during the day rather than at night and at about one hour after exercise. There is a slight to moderate elevation of serum potassium and increased reactivity to acetylcholine. Attacks of weakness may be precipitated by oral potassium administration, prevented by the prior administration of glucose, and relieved by the intravenous administration of calcium.

Weakness resulting from hyperkalemia clinically resembles that resulting from hypokalemia, but the electromyographic and electrocardiographic alterations are different. Since the administration of potassium to normal subjects causes an increase in reactivity of muscle to the depolarizing action of acetylcholine, the weakness of hyperkalemia may be attributable, at least in part, to a decrease in muscle-membrane potential (depolarization), which, in turn, is attributable to a decrease in the concentration gradient of potassium (28).

Calcium.—There is evidence that calcium is necessary for development of the "active state" of contraction of skeletal, cardiac, and smooth muscle (109). The ion enters the muscle fiber during or following depolarization (110), leaves during contraction (111), and may serve as the link between the two processes. Nevertheless, diminution in the concentration of ionized calcium in the plasma below normal results in increased irritability and spontaneous discharge of sensory and motor nerves and of muscle, producing "tetany." Increase in calcium above 12 mg. per cent has an opposite effect and results in hypotonia and weakness, which is attributable to decreased muscle excitability and contractility (112). Hypercalcemia also causes decreased activity of smooth muscle resulting in constipation, anorexia, nausea, and vomiting.

Magnesium.—Reduction in the plasma concentration of magnesium is reported to cause muscle spasm and convulsions resembling tetany, probably because of increased excitability of nerve and muscle. Increase in plasma magnesium concentration following administration of large doses of magnesium salts produces muscular weakness resulting from decreased

muscle excitability and contractility (112), reduction in blood pressure resulting from vasodilatation, and depression of the central nervous system.

Adrenal Hormones

Epinephrine.—This compound reduces muscle fatigue, apparently by increasing the output of acetylcholine from nerve endings (113). Epinephrine, norepinephrine and isoprenaline are also reported to increase twitch tension in slow-contacting muscle such as the cat soleus (114).

Adrenal steroids.—Muscular weakness is a common finding in hyperadrenocorticism. Severe weakness and wasting of the muscles of the pelvis and lower extremities may also take place after prolonged administration of large doses of 17-hydroxycorticosteroids (115). There is increased creatinuria and minimal histologic change. Potassium administration does not affect the weakness or wasting, but discontinuation of steroid is followed by return to normal. The myopathy is believed to be due to altered protein metabolism, but the mechanism is not clear.

Fatigue

Following repetitive nerve stimulation, fatigue of neuromuscular transmission occurs as a result of decreased sensitivity of the endplate to the acetylcholine, increased threshold of the muscle fiber for excitation, and decreased output of acetylcholine from the motor-nerve endings (116). There is difference of opinion concerning the relative rate of fatigue of the contractile mechanism. The nerve trunk is practically indefatigable.

Alteration in Temperature

The optimal muscle temperature for maintaining sustained contraction is reported to be 25 to 29°C. in man (117, 118). In the rat, the maximum twitch tension is attained at 20° in innervated muscle and at 39° in denervated muscle (119). Lowered temperature increases the magnitude and duration of the neuromuscular blocking action of depolarizing agents such as decamethonium, and decreases the blocking action of *d*-tubocurarine (120). It also increases the contractural response to acetylcholine and decreases that to potassium (119).

DISEASE STATES

Myasthenia Gravis

Defect in neuromuscular transmission.—This has many of the characteristics of a nondepolarizing and competitive (acetylcholine-inhibitory) block, similar to that produced by *d*-tubocurarine in normal subjects. In myasthenic patients the block appears to be produced by acetylcholine released in a normal manner during neuromuscular transmission, or by choline or a closely related compound formed following hydrolysis of the transmitter. The intraarterial administration of either acetylcholine or

choline produces in most myasthenic patients a neuromuscular block that inhibits the depolarizing action of acetylcholine and is reversed by acetylcholine or anticholinesterase compounds (47). This competitive and presumably nondepolarizing block is in contrast to the predominantly noncompetitive block produced by these compounds in normal subjects, in whom the impairment is intensified by acetylcholine or anticholinesterase compounds (47). The latter block would appear to be of the depolarizing type, but confirmation of such a mechanism requires measurement of membrane potential, which has only recently been made in normal man (121) and has not yet been applied to the study of disease or drug action. The relationship of these functional changes to abnormalities which have been described in the distal nerve endings and motor end-plates of myasthenic muscle (122, 123, 124) is not clear. A contrasting view of the defect in myasthenia is that it is caused by impaired release of acetylcholine from motor-nerve endings, as suggested by the resemblance between the posttetanic exhaustion of myasthenic muscle and of normal muscle treated with hemicholinium (125).

Different types of neuromuscular block in myasthenia gravis: the acetylcholine-insensitive state.—In some myasthenic patients the block produced by the intraarterial injection of acetylcholine or choline inhibits the depolarizing effect of acetylcholine, but is not reversed by this compound or anticholinesterase compounds (126). Such a block, which resembles one stage of the effect of acetylcholine in the frog (27, 30), may be termed acetylcholine-insensitive. Patients who manifest this type of block respond poorly or not at all to anticholesterase medication; they are clinically acetylcholine- and neostigmine-insensitive. Patients in whom acetylcholine produces the more usual acetylcholine-reversible block may become acetylcholine-insensitive during an exacerbation of their disease, or they may be rendered acetylcholine-insensitive by the repeated intraarterial administration of this compound or choline, or by prolonged repetitive nerve stimulation (126), which probably causes the local accumulation of endogenous acetylcholine or choline. It is this insensitive state that constitutes the main problem in management of the disease and often leads to the administration of large doses of anticholinesterase medication and to confusion as to whether the patient is overdosed (in "cholinergic crisis") or underdosed (in "myasthenic crisis"). Many patients who are thought to be overdosed are really in an insensitive state, and large doses of anticholinesterase compound often render the patient more insensitive (37).

While it is possible that the neuromuscular blocks occurring in myasthenic patients may be a result of abnormal products of acetylcholine or choline capable of producing a competitive or acetylcholine-insensitive block in normal subjects, it seems more likely that they are due to abnormal responses of the motor end-plates to substances normally released from the motor-nerve endings (126). Myasthenic patients react abnormally not only

to acetylcholine and choline, but also to many other quaternary ammonium compounds, including decamethonium (47, 127, 128) and succinylcholine (129). In normal subjects, decamethonium block of moderate degree does not inhibit the depolarizing action of acetylcholine and is not reversed by it or anticholinesterase compounds, whereas decamethonium block of marked degree does inhibit the action of acetylcholine and is reversed by acetylcholine or anticholinesterase compounds. In myasthenic patients, all levels of decamethonium block are reversed by these compounds.

Anticholinesterase compounds used in the management of myasthenia gravis.—The most useful of these are the quaternary ammonium compounds, neostigmine, pyridostigmine (Mestinon), and ambenomium (Mytelase). Bis-neostigmine (BC-40) (38) and bis-pyridostigmine (BC-51, hexamarium) (130) are longer acting quaternary ammonium anticholinesterase compounds which require less frequent administration, but are less suitable for general use because of the danger of cumulation and overdose. Several organophosphorus anticholinesterase compounds have also had clinical trials in the management of myasthenia gravis. These have included DFP, TEPP, HETP, OMPA (131), echothiophate (42), and sarin (37). While these compounds are more prolonged in their action than the quaternary ammonium compounds and produce a more even and sustained increase in strength following the administration of only one or two doses a day, the danger of cumulation and overdose has precluded their general clinical use except under carefully controlled circumstances. The maximal strength obtained after optimal doses of any of these quaternary ammonium or organophosphorus anticholinesterase compounds is approximately the same (37). The compounds differ mainly in their duration of action (organophosphorus compounds > bis-neostigmine and bis-pyridostigmine > pyridostigmine and ambenomium > neostigmine) and in the severity of their parasympathomimetic side effects (organophosphorus compounds > bis-neostigmine and bis-pyridostigmine > neostigmine > pyridostigmine and ambenomium). Recently anticholinesterase compounds have also been administered by local instillation in patients with ocular myasthenia (132).

Management of overdose of anticholinesterase compound.—In the management of myasthenia gravis, graded doses of an anticholinesterase compound are administered until a maximal level of strength is attained in the affected muscles. Unfortunately, in patients with severe myasthenia the maximal strength attained may be far below normal. Increasing doses may result in no further increase in strength, and excessive drug may produce generalized weakness, attributable to the accumulation of an excessive concentration of acetylcholine at the motor end-plates and prolonged depolarization of this region, followed by refractoriness of the end-plates to acetylcholine (126). If overdose is suspected, the simplest procedure is to withhold medication for several hours and carefully observe whether the

strength increases or decreases. It is frequently helpful to observe the effect on the weakness of intravenous administration of the short-acting anticholinesterase compound, edrophonium, or of an oxime. The oximes 2-PAM, DAM, and TMB-4 are capable of reversing neuromuscular block produced by anticholinesterase compounds in myasthenic patients, as in normal subjects (62). The effect of this reversal depends on the prior action of the anticholinesterase compound, since neuromuscular function is restored toward its initial state. Whereas in normal subjects neuromuscular function and strength are returned toward normal, in myasthenic patients the effect depends on the status of the patient at the time of oxime administration. If sufficient anticholinesterase compound was administered to depress function, this is restored to a more optimal level, but if function was optimal at the time of oxime administration, it is restored toward the basal level present prior to the administration of anticholinesterase compound; i.e., it is depressed. It is therefore recommended that myasthenic patients suffering from anticholinesterase intoxication be titrated with successive doses of oxime until strength is restored to the maximal level attained following the administration of optimal doses of anticholinesterase compound.

Unfortunately, many patients with severe myasthenia who are responding poorly to anticholinesterase medication fail to improve following either expectant management or administration of oxime or edrophonium, and they may become worse after any of these measures. The main difficulty in these patients is neither insufficient nor excessive anticholinesterase medication, but a refractory or "insensitive" state of the motor end-plates to the normal depolarizing action of acetylcholine (126). The only therapeutic measure available for this condition is to maintain respiration and reduce the dosage of anticholinesterase medication until response to this medication improves (37). In one patient, prolonged administration of *d*-tubocurarine, with artificial respiration, was followed by improvement in clinical state and in responsiveness to anticholinesterase medication, presumably as a result of the complete rest afforded the end-plates (133). This observation is of interest, but the procedure is too hazardous for general use.

Myasthenic Syndrome With Carcinoma

Some patients with neoplasm, particularly small cell bronchogenic carcinoma, have muscular weakness and fatigue, most marked in the extremities (134). The syndrome resembles myasthenia gravis in symptomatology and, usually, in increased reactivity to *d*-tubocurarine and abnormal reactivity to decamethonium and succinylcholine. It differs from myasthenia gravis in that only a minority of patients respond to neostigmine (135). Furthermore, the action potential and twitch evoked by a maximal nerve stimulus is much lower in relation to the strength of voluntary contraction than in myasthenia gravis, and the degree of facilitation during or after repetitive nerve stimulation is much greater. In some patients, weakness

has appeared many months before the tumor was noted, while in others it has followed the tumor. In some patients evidence of peripheral neuropathy has occurred in the presence of neoplastic disease.

Denervation

The motor nerve appears to exert a direct restraining influence on the chemo-sensitivity of muscle, and removal of this influence is followed by supersensitivity to acetylcholine. Denervation is followed by an increase in the chemo-sensitive area beyond the end-plate region, with no increase in the sensitivity of the receptors themselves (136). A similar increase in the chemo-sensitive area occurs following exposure of muscle to botulinus toxin (137). Since this toxin prevents transmitter release from motor-nerve endings, it appears that this release determines the size of the acetylcholine-sensitive area and that the effect of denervation is caused by interruption of transmitter release. Supersensitivity to acetylcholine has also been reported in mice with hereditary myopathy (138) and in rabbits with vitamin-E-deficient myopathy (139), and has been attributed to local functional denervation.

Spastic States

Unless facilities for endotracheal intubation and artificial respiration are available, neuromuscular blocking agents such as *d*-tubocurarine are too hazardous for use in the relief of muscle rigidity or spasm. Centrally acting agents are used for this purpose, in spite of their relatively low efficacy. In parkinsonism, up to 25 per cent improvement in muscle rigidity and tremor can be achieved by the use of anticholinergic drugs such as belladonna alkaloids, trihexyphenidyl (Artane), cycrimine hydrochloride (Pagitane), procyclidine hydrochloride (Kemadrin), benztropine methanesulfonate (Cogentin), or orphenadrine (Disipal) (140). Diphenhydramine (Benadryl) may have some value as an adjunct to these drugs. Chlorpromazine (Thorazine) and Rauwolfia alkaloids are of no practical value, and may occasionally produce a parkinsonian-like state. Muscle spasticity, whether central or peripheral in origin, may be temporarily alleviated by the intravenous administration of mephanesin (Myanesin) or its congener methocarbamol (Robaxin), but is usually little affected by the oral administration of these drugs, or meprobamate (Equanil, Miltown) or zoxazolamine (Flexin).

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CARDIOVASCULAR PHARMACOLOGY[1,2]

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CARDIAC GLYCOSIDES

Considerable attention has been devoted in recent years to the actions of cardiac glycosides on myocardial contractility and the peripheral vascular system. Most of this interest has centered upon descriptions of the positive inotropic action of cardiac glycosides in both the presence and absence of failure, upon the electrolyte composition of heart muscle, and upon the energy sources for muscular contraction. These investigations have led to an increased understanding of the hemodynamic actions of glycosides and of the actions of glycosides on biochemical mechanisms.

Cardiac Actions

It has been repeatedly claimed that glycosides either depress or at least do not stimulate the normal heart. For example, Olson, Roush & Liang (1) concluded that acetyl strophanthidin had a negative inotropic effect in the normal dog, since cardiac output and calculated cardiac work were decreased promptly following injection of approximately 55 per cent of the fatal dose of the glycoside. In dogs with congestive heart failure, on the other hand, the same dose of acetyl strophanthidin increased cardiac output and ventricular stroke work. Regan, Talmers & Hellems (2) also reported that acetyl strophanthidin had a negative inotropic action in the normal dog, since cardiac output and left ventricular stroke work diminished after administration of the glycoside. Mercier and co-workers (3) found that ouabain and digoxin decreased ventricular work despite a rise in arterial pressure in dogs anesthetized with chloralose. Similarly, it had been reported that the papillary muscle of the cat failed to respond to nontoxic concentrations of several glycosides, although the same concentrations of these compounds increased the contractile force of failing muscle [White & Salter (4); Sciarini, Ackerman & Salter (5)]. In contrast to the foregoing papers, numerous recent investigations have shown that cardiac glycosides increase the force of myocardial contraction of normal heart muscle equally as well as that of failing heart muscle. One of the most definitive studies of this problem is that of Sanyal & Saunders (6) who used strips of guinea pig

[1] The survey of the literature pertaining to this review was concluded in August, 1960.

[2] Abbreviations used in this chapter include: DCI (dichloroisoproterenol); DOC (desoxycorticosterone); EDTA (ethylenediaminetetraacetic acid); PC (creatine phosphate); IP (inorganic phosphate); P:O ratio (ratio between the uptake of inorganic phosphate and uptake of oxygen); TMA (tetramethylammonium).

ventricular muscle driven by rhythmic electric stimuli. Ouabain increased the contractile force substantially before any evidence of failure was observed, as well as after failure had been produced by permitting the muscle to contract in the presence of a phosphate buffer. When a bicarbonate buffer was employed, muscle preparations showed no evidence of failure over periods of up to seven hours, and ouabain increased the contractile force substantially whether added promptly after establishing the preparation or after several hours had elapsed. These latter results were confirmed by the investigations of Stewart (7) who showed that the contractile amplitude of guinea pig ventricle strips contracting in the presence of a bicarbonate buffer was increased by cardiac glycosides. The positive inotropic action of cardiac glycosides has also been demonstrated in intact, normal animals, including human beings. In anesthetized normal dogs ouabain, in a dose approximating 40 per cent of the fatal dose, increased the force of ventricular contraction as measured with a strain-gauge arch [Cotton & Stopp (8)]. In these same experiments, ouabain also increased the amount of ventricular stroke work performed at any given ventricular filling pressure over a wide range of filling pressures. These findings confirmed the earlier work of Walton, Leary & Jones (9), who found that a number of glycosides all increased ventricular contractile force of normal dogs to about the same extent when administered in equivalent fractions of their fatal doses. In addition to normal experimental animals, human subjects without clinical evidence of heart failure also respond to acetyl strophanthidin with a marked increase in ventricular contractile force as measured with a strain-gauge arch [Bloodwell *et al.* (10)]. The characteristics of the contractile response in human subjects were the same as those observed in dogs and other mammalian species. Although glycosides stimulate both normal and failing cardiac muscle alike, failing heart muscle may be more sensitive to glycosides. Olson, Roush & Liang (1) and Bliss & Adolph (11) have shown that dogs with experimental heart failure developed cardiac arrhythmias with doses of acetyl strophanthidin having no effect upon the cardiac rhythm of normal dogs. There are no quantitative data available, however, regarding the sensitivity of failing and normal heart muscle with respect to the action of glycosides upon myocardial contractility.

Glycosides increase not only the contractile force of heart muscle, but of skeletal and arterial smooth muscle as well. Faust & Saunders (12) have clearly shown that the contractile force of the unfatigued isolated diaphragm from the guinea pig was increased by ouabain when the muscle was stimulated both directly and indirectly with supramaximal shocks. The increase in the contractile force of the isolated diaphragm occurred with the same concentrations of ouabain used to increase the contractile force of ventricle strips from the same animals. These findings are in contrast to much previous work showing that glycosides do not increase the force of contraction of skeletal muscle, but consideration of the methods employed by Faust & Saunders (12) leaves little doubt that cardiac glycosides augment the contractility of skeletal as well as cardiac muscle. The contractile

strength of strips of carotid artery from the rabbit is also increased by cardiac glycosides [Leonard (13)].

Temperature is an important determinant of the magnitude of the contractile response to glycosides. Lowering the body temperature of anesthetized dogs from 37° to 22 to 25° caused a marked reduction in the positive inotropic action of ouabain as compared with responses obtained in these same animals at normal body temperature [Cotten & Brown (14)]. Under these conditions, sympathomimetic amines such as norepinephrine still produced substantial increments in ventricular contractile force at a time when the response to ouabain was virtually abolished. Saunders & Sanyal (15) found a direct relationship between temperature and the magnitude of the positive inotropic response to ouabain. In these experiments with guinea pig ventricle strips, ouabain increased the contractile force substantially at a bath temperature of 37°, but the contractile response diminished markedly as the temperature was reduced to 27°; at 17° ouabain failed to affect the contractile force. However, the significance of these findings is clouded by the fact that the control level of contractile force increased progressively as the bath temperature was reduced, being approximately 90 mg. at 37° and 250 mg. at 17°. The possibility thus exists that the positive inotropic action of ouabain was simply masked at low temperatures by the rise in the control contractile force to levels far above the maximum force produced by ouabain at 37°. The fact that rate of onset of the positive inotropic action of ouabain was not signficantly affected by lowering the bath temperature led Saunders & Sanyal (15) to suggest that the rate-determining process resulting in the onset of the contractile response to ouabain is of a physical rather than a chemical nature. The rate of onset of action of ouabain is dependent, however, upon the frequency of contraction as has been demonstrated by Sanyal & Saunders (16) using strips of ventricular muscle from guinea pigs. As the frequency of contraction was increased progressively, the time required for onset of action of the positive inotropic effect of the glycoside was progressively shortened. At a contraction frequency of 200/min. the maximum contractile force response to 2.3×10^{-7} *M* ouabain occurred in approximately 10 minutes, while at a contraction frequency of 25/min. the maximum effect required about 40 minutes to develop. Extrapolation of these data led Sanyal & Saunders (16) to the conclusion that ouabain produces the effects leading to an increased force of contraction only in actively contracting muscle. If this conclusion is a valid one, as it now appears, the significance of much previous work done with noncontracting muscle in experiments aimed at elucidating the biochemical actions of glycosides is subject to serious question.

The maximum force achieved in response to ouabain was influenced by the frequency of contraction in the experiments of Sanyal & Saunders (16) described above. At a frequency of 25/min., the control contractile force averaged 45 mg., and ouabain increased the force to an average maximum of 90 mg., an increase of 100 per cent. At a contraction frequency of 100/min., on the other hand, the control contractile force averaged 110 mg.,

and ouabain increased the force to an average maximum of 162 mg., an increase of only 48 per cent. Thus, as the frequency of contraction was increased, the per cent response to ouabain was decreased markedly because of the elevated control level of contractile force, whereas the actual increment in force evoked by ouabain was relatively unaffected.

Peripheral Vascular Effects

Although the preponderance of evidence indicates that cardiac glycosides increase the contractile force of normal and failing heart muscle alike, an explanation is needed for the failure of glycosides to augment the cardiac output despite an increase in contractile strength. This problem has been resolved, at least in part, by the finding that glycosides decrease venous return in normal dogs concomitantly with the increase in myocardial contractility [Cotten & Stopp (8)]. In these experiments ouabain increased myocardial contractility and blood pressure but decreased cardiac output, left atrial pressure, and heart rate of normal anesthetized dogs. When left atrial pressure was kept constant throughout the action of the glycoside by controlled infusion of blood into the left atrium, cardiac output was substantially increased as was left ventricular stroke work. Venous return was decreased by approximately 15 ml./kg. in these experiments as judged by the amount of infused blood required to keep left atrial pressure constant while the effects of ouabain developed. These findings have been confirmed by Ross, Braunwald & Waldhausen (17) who found that acetyl strophanthidin reduced venous return by about 13 ml./kg. in dogs in which the direct cardiac actions of the glycoside were excluded through the use of the cardiopulmonary bypass technique and a constant perfusion rate. The decrease in venous return is apparently caused by venous pooling of blood, especially in the splanchnic bed, since venous return actually increased by about 8 ml./kg. when the splanchnic bed was excluded from the circulation prior to administration of acetyl strophanthidin. The exact mechanisms responsible for the trapping of blood in the splanchnic bed have not yet been elucidated, although venoconstriction probably plays an important role. Ross, Braunwald & Waldhausen (17) noted an increase in pressure in the inferior and superior venae cavae after administration of acetyl strophanthidin at a time when venous return was reduced indicating that venoconstriction had taken place. The increase in venous return when the splanchnic bed was excluded from the general circulation offered a further indication of a generalized venoconstrictor action of the glycoside. A venoconstrictor action of cardiac glycosides has also been demonstrated in human subjects by Horsley & Eckstein (18) who measured venous tone in the forearm veins. Evidence of a venoconstrictor effect of glycosides on hepatic smooth muscle in normal human subjects has been obtained by Baschieri *et al.* (19). In the latter study, digitalization was followed by an increase in hepatic wedge pressure, an increase in the gradient between the hepatic wedge pressure and the inferior vena caval pressure, and a reduction in hepatic blood flow. Cardiac glycosides have also been shown to

have a marked constrictor action on arterial smooth muscle. Ross, Waldhausen & Braunwald (20), using dogs subjected to the cardiopulmonary bypass technique, found that acetyl strophanthidin, ouabain, and lanatoside C each increased arterial pressure substantially when a constant perfusion rate was used. The rise in pressure reflected an increase in peripheral resistance produced by the glycosides. The elevation of the peripheral vascular resistance was not abolished by ganglionic blockade or by adrenalectomy. Studies with human subjects undergoing total cardiopulmonary bypass with a constant perfusion rate have yielded equivalent results upon injection of acetyl strophanthidin [Bloodwell *et al.* (10)]. This increase in total peripheral resistance and consequent rise in blood pressure is, of course, not commonly observed when patients are digitalized slowly, but may be readily demonstrated when a glycoside is given by rapid intravenous injection [Williams, Zohman & Ratner (21)].

Effect of Glycosides on Electrolyte Composition of Heart Muscle

Increasing interest is developing in the influence of glycosides on the electrolyte composition of cardiac muscle. These investigations deserve particular attention in view of the marked influence which alterations in ionic content and composition exert on the contractile process. Glycosides increase the influx of calcium[45] into isolated, rhythmically contracting rabbit atria, but have no apparent effect on its efflux [Holland & Sekul (22)]. The influx of calcium was markedly increased by ouabain when the concentration of potassium in the medium was low, but was severely reduced when potassium concentration was elevated. These results suggest that at least part of the positive inotropic action of glycosides may be related to the effects of increased calcium either at the cell membrane or in the myoplasm. Such an hypothesis is consonant with a considerable body of earlier work showing that the magnitude of glycoside action upon myocardial contractility of isolated cardiac muscle can be enhanced by increasing the concentration of calcium in the medium, and decreased in the presence of a calcium deficiency [for discussion and reference see (23)].

In addition to promoting an increase in the influx of calcium, glycosides also cause a negative potassium balance in heart muscle (24 to 27). Rayner & Weatherall (24) have shown that digoxin and ouabain reduced the influx of potassium into isolated, beating rabbit auricles, but had no appreciable effect on the efflux of potassium, leading to a net decrease in intracellular potassium content. The loss of potassium was accompanied by a gain of sodium. Similar changes in intracellular potassium of the guinea pig heart were obtained by Vick & Kahn (25) and by Vick (26) who also observed that the loss of potassium was small at concentrations of ouabain producing a positive inotropic action, but increased as the concentration of ouabain was increased. Loss of potassium from heart muscle after administration of cardiac glycosides to intact animals is a well-established phenomenon [see Cairns, Love & Burch (27)]. Glycosides also inhibit the influx of potassium into human red cells bringing about a net fall in intracellular

potassium [Glynn (28); Gill & Solomon (29)]. This effect may be attributable to an action of the glycosides on the red cell transport system for potassium rather than the cell's energy supply, since there were no changes observed in ATP, ADP, or 2,3-diphosphoglyceric acid [Kunz & Sulser (30)]. The action of potassium on cell membranes of heart muscle and red blood cells may involve similar mechanisms, but the validity of this assumption remains to be proved.

The exact relationship of the reduction in intracellular potassium to the positive inotropic action of cardiac glycosides remains obscure. Hajdu (31) has suggested that the positive inotropic action of glycosides is caused by reduced re-entry of potassium into cardiac cells during relaxation providing a more optimal intracellular environment for contraction of the contractile proteins. This hypothesis was based upon Hajdu's finding that reduction in intracellular potassium by 3 m.eq./l. of fiber water from a level of 107 m.eq./l. changed the contractile force from zero to maximum values. However, it would appear from the work of Vick (26) that potassium loss from myocardial cells per se may not be a causative factor in the glycoside-induced augmentation of contractile force, since several lactones having a negative inotropic action also caused a loss of intracellular potassium. In these same experiments ouabain caused a similar loss of potassium but had a positive inotropic action.

The development of contracture in isolated heart muscle after exposure to toxic amounts of cardiac glycosides may be related, at least in part, to the loss of potassium, as had been suggested by Vick & Kahn (25) and by Vick (26). In their experiments the isolated guinea pig heart lost potassium when contracture was progressing. With the disappearance of contracture, on the other hand, the heart gained potassium. Recent work by Thomas (32) demonstrated the role of calcium in the production of contracture. Development of contracture in the frog heart treated with ouabain was associated with an increased uptake of calcium[45]. Administration of ethylenediaminetetraacetic acid (EDTA) abolished the contracture induced by ouabain, but not that induced by iodoacetate. Thomas (32) interprets his findings to mean that contracture induced by ouabain is chiefly attributable to altered handling of calcium by the cells and not primarly to a shift in the sodium or potassium concentration or to gross interference with intracellular energy metabolism.

Metabolic Actions of Glycosides

The possibility that the positive inotropic response to cardiac glycosides might be explained in terms of associated metabolic changes has led to numerous studies. Most of these investigations have dealt with the actions of glycosides upon labile energy-rich phosphate compounds and upon carbohydrate metabolism. Only a few of the more recent papers are discussed in detail below.

Furchgott & de Gubareff (33) have demonstrated that concentrations of K-strophanthin inducing a maximum increase in contractile amplitude of

the electrically driven, hypodynamic left atrium from the guinea pig have no demonstrable effect on the concentration of ATP, ADP, and creatine phosphate (PC) in the muscle. High concentrations of K-strophanthin inducing manifestations of cardiac toxicity, on the other hand, resulted in significant reductions in the concentration of ATP and PC, while increasing the concentrations of ADP, AMP, and inorganic phosphate (IP). These observations have been confirmed and extended by Lee, Yu & Burstein (34) who simultaneously measured changes in contractile force, oxygen consumption, and concentrations of ATP and PC in the isolated cat papillary muscle. In this study a relatively high concentration of ouabain (1.37×10^{-6} *M*) increased the contractile force some seven or eight minutes before oxygen consumption began to increase. At this time there were no significant changes in the concentrations of ATP or PC. The increase in contractile force without a concomitant change in oxygen consumption led to a substantial increase in the mechanical efficiency of the papillary muscle. The maximum increase in contractile strength was obtained within approximately 40 minutes after addition of ouabain to the bath. At this time the oxygen consumption had increased to nearly the maximum value obtained in any given experiment, but the concentrations of ATP and PC were decreased only slightly. Some 80 minutes after addition of ouabain to the bath contracture developed, and little or no contractile tension was detectable. Oxygen consumption continued at the maximum value during contracture despite the loss of individual contractions, and the concentrations of ATP and PC were significantly reduced. Using a lower concentration of ouabain (2.74×10^{-7} *M*), Lee, Yu & Burstein (34) were able to show a marked temporal separation of about 20 minutes between the time of onset of the positive inotropic effect and the time of onset of the increase in oxygen consumption. The increased oxygen consumption seen after administration of ouabain to the cat papillary muscle apparently is not the result of the increased contractile force per se, because experiments performed by Lee and co-workers (34) with both resting and contracting cat papillary muscles demonstrated that approximately the same changes in oxygen consumption and concentrations of ATP and PC occurred upon the addition of 1.37×10^{-6} *M* of ouabain. The times required for attainment of the maximum increases in oxygen consumption and decreases in concentrations of ATP and PC were longer for the resting muscle than for the contracting muscle.

The observation that cardiac glycosides increase the oxygen consumption of heart muscle, but not that of other tissues has been reconfirmed recently by Peschel & Schlayer (35). The basis for the increased oxygen consumption by heart muscle after exposure to a glycoside remains uncertain. One possible explanation has been suggested by Lee and his co-workers (34), but the evidence is, at present, circumstantial. An increase in the content of ADP and IP has been shown to accelerate oxidation steps coupled with phosphorylation when these substances are limiting [Lardy & Wellman (36); Hock & Lipmann (37)]. Since it has been shown that the content of

ADP and IP were increased when guinea pig atrial muscle was exposed to excessive amounts of a cardiac glycoside (33), part of the increased oxygen consumption may be related to these changes. Another possible explanation for the increased oxygen consumption evoked by glycosides has been suggested by Lee, Schwartz & Burstein (38) who have presented some evidence for a mild glycoside-induced uncoupling of oxidative phosphorylation. As has been amply demonstrated, glycosides have no effect upon the oxygen uptake or the ratio between the uptake of inorganic phosphate and uptake of oxygen (P:0 ratio) of mitochondria from heart muscle either when the glycoside is added *in vitro* or given *in vivo* [Lee, Schwartz & Burstein (38); Plaut, Gertler & Plaut (39)]. However, in the presence of a deficiency of IP or phosphate acceptor (ADP), oxygen uptake of mitochondria from atrial muscle of guinea pigs pretreated with a large dose of ouabain or digitoxin was significantly greater than that of mitochondria from untreated animals [Lee, Schwartz & Burstein (38)]. Further, in the presence of excess IP the addition of hexokinase and glucose, to increase the amount of available ADP, increased the oxygen uptake of mitochondria from the untreated animals but had no effect upon the already elevated oxygen uptake by the mitochondria from the animals pretreated with a glycoside. The increased oxygen consumption of mitochondria from the treated animals was found not to be attributable to an activation of ATPase. These data permit the tentative conclusion reached by Lee, Schwartz & Burstein (38) that glycosides induce mild uncoupling of oxidative phosphorylation. The increased oxygen uptake seen with heart mitochondria from animals pretreated with a glycoside is presumed to be related to an undemonstrated increase in intramitochrondrial IP or ADP, or both. However, final proof of an uncoupling action by glycosides will require a demonstration of a reduced P:0 ratio in the presence of an IP or ADP deficiency, and failure of this deficiency to increase upon addition of hexokinase and glucose in the presence of excess IP. Whatever the explanation for the increased oxygen consumption, it does not appear to be the result of the increased contractility per se, since oxygen consumption increases whether the muscle is contracting or not (34). Neither does an increased oxygen consumption per se lead necessarily to an increased vigor of contraction. Addition of 20 m*M* succinate to a substrate-free medium containing a contracting papillary muscle increased oxygen consumption to approximately the same extent as produced by 2 m*M* of pyruvate, but had no effect on the contractility [Lee, Yu & Burstein (34)]. Similarly, increasing the concentrations of ATP and PC by the addition of succinate did not lead to an increase in contractile force. It would thus appear that the positive inotropic action of glycosides is not dependent upon an increase in the content of energy-rich phosphates in the heart, a conclusion upon which there is general agreement [see Furchgott & de Gubareff (33) and Wollenberger (40)]. Further, the increased contractile force and augmented oxygen consumption produced by glycosides does not seem to be dependent upon increased substrate uptake caused by altered membrane permeability, since the characteristic effects of ouabain on force

and oxygen consumption are evoked in a substrate-free medium [Lee, Yu & Burstein (34)]. There is some evidence, however, that ouabain may increase glucose utilization, since the positive inotropic effect of glucose on rat ventricle strips is enhanced in the presence of ouabain [Berman, Masuoka & Saunders (41)]. Further, Kien & Sherrod (42) have reported that "therapeutic" doses of digoxin (.065 mg./kg.) increased the rate of C^{14}-labelled glucose utilization by the myocardium by about 60 per cent, and increased the contribution of glucose to the total myocardial metabolism which, however, was unchanged. The increased turnover of the labelled glucose was reflected in a marked increase in the production of $C^{14}0_2$ by the myocardium. The significance of these observations in relation to the stimulant action of glycosides on myocardial contractility is obscure, however, since Kien & Sherrod (42) also reported that the dose of digoxin which they employed had no demonstrable effect upon the cardiovascular system. The absence of changes, especially in systemic arterial pressure and cardiac output, in the normal dogs used is suprising in view of the ample demonstrations that glycosides (including digoxin) have distinct cardiovascular effects when given in the same fraction of the fatal dose used by Kien & Sherrod (42). In any event, the possibility that glycosides correct a specific metabolic lesion in failing heart muscle, as discussed by Kien & Sherrod (42), appears to be remote judging from the excellent studies of Furchgott & deGubareff (33) and Lee, Yu & Burstein (34) on energy metabolism, and from the ample demonstrations that glycosides augment myocardial contractile force whether the heart muscle is normal or in failure.

Structure-Activity Relationships

The cardiotonic effects of various substances having structural similarities to cardiac glycosides have been examined by several groups of investigators. The simple lactones, α-angelicalactone and pulvinic acid dilactone, both have been shown to exert a negative inotropic effect upon the hypodynamic isolated guinea pig ventricle [Vick (26)]. This finding is in keeping with the earlier work by other investigators [for references see (23)]. On the other hand Vick (26) found that Patulin, a steroid lactone consisting of an α, β-unsaturated γ lactone attached to a single ring, increased briefly the amplitude of contraction when high concentrations were employed. In these same experiments both ouabain and dihydro-ouabain had positive inotropic effects, the magnitude of which were proportional to the concentrations used. The data suggest that lactones per se do not possess the same pharmacological properties exhibited by cardiac glycosides, but that coupling a simple lactone to a ring structure confers cardiac stimulant properties upon the molecule. Much additional work is required to validate this hypothesis, however.

Bennett *et al.* (43) studied the effects of a large series of conjugated carbonyl compounds on the contractility of the cat papillary muscle. These compounds are closely related to the opened lactone ring of the cardiac glycosides. Acrolein, methylvinylketone, and 4-acetoxy-4-hydroxy-2-pen-

tenoic acid γ lactone were among the more active compounds examined. These substances were capable of restoring the contractile amplitude of the hypodynamic papillary to control levels. The action of some substances persisted for long periods, and contracture could be produced by a sufficiently high concentration. The significance of the data is partially obscured by the finding that the compounds produced a positive inotropic action only in the hypodynamic cat papillary muscle, and not in the nonfailing muscle. This is in contrast to the action of cardiac glycosides such as ouabain.

Various steroids have also been found to evoke changes in the contractile activity of heart muscle. For example, desoxycorticosterone (DOC) and progesterone are effective in abolishing the staircase phenomenon in the isolated beating frog heart, an action similar to that evoked by strophanthidin [Hajdu (44)]. Hajdu also found that corticosterone had an action similar to that evoked by DOC, but was much weaker. Aldosterone had no effect on the staircase phenomenon, whereas steroids such as cortisone, hydrocortisone, and 17-hydroxy progesterone, all having hydroxyl groups in the 17 position, had an effect opposite to that of DOC and strophanthidin. The steroid 9-α-fluorohydrocortisone has a positive inotropic effect associated with a loss of intracellular potassium and a gain of sodium [Tanz, Whitehead & Weir (45)]. In high concentrations, however, 9-α-fluorocortisone depresses myocardial contractility, and the muscle gains potassium and loses sodium. Cortisone also exerts a marked action on the isolated failing heart when added in small quantities, restoring the amplitude of contraction to control levels and protecting the histologic integrity of the muscle [Tanz (46)]. Estrogen [King *et al.* (47)] and testosterone, norethandrolone, and progesterone [Van Arman & Drill (48)] all appear to have negative inotropic actions on isolated heart muscle.

In addition to the direct actions of certain steroids on myocardial contractility some evidence exists to show that certain steroids may modify the actions of glycosides. Grinnell & Smith (49) demonstrated that normal female dogs in anestrus or estrus and castrate female dogs treated with estrogen were much more resistant to the development of cardiac arrhythmias produced by a large dose of digoxin (0.15 mg./kg.) as compared with male dogs or nontreated castrate females. These experiments involved only recordings of cardiac arrhythmias so that no information is currently available concerning a possible interaction of glycosides and estrogen on myocardial contractility. Adrenalectomized rats appear to be more sensitive to ouabain than normal rats with respect to the development of conduction disturbances [Unterman, de Graff & Kupperman (50)], but the basis for the increased sensitivity is unkown. It is also unknown whether adrenalectomy alters the sensitivity of the contractile mechanism to cardiac glycosides.

DRUGS WHICH AFFECT THE CARDIOVASCULAR SYSTEM BY ACTING ON THE SYMPATHETIC NERVOUS SYSTEM

The discovery of new types of pharmacological antagonists of adrenergic stimulant substances, and studies aimed at correlating the physiologi-

cal effects of adrenergic stimulant agents with their metabolic actions have provided new insight into the physiological functions of the sympathetic nervous system and the interaction of adrenergic stimuli on biochemical processes. This portion of the review will be limited to an examination of a few significant contributions in these areas. A more general review of the cardiovascular effects of sympathomimetic amines is that by Aviado (51).

Adrenergic Drugs

The concept of adrenotropic receptors.—The classification of adrenotropic (adrenergic) receptors first proposed by Ahlquist (52) has received substantial verification through the discovery of the special adrenergic blocking properties of dichloroisoproterenol (DCI) by Powell & Slater (53). Ahlquist (52, 54) had proposed that receptors in the adrenergic system be classified as *alpha* and *beta, alpha* receptors subserving primarily excitatory effects of adrenergic stimuli except for those on the heart, and *beta* receptors subserving inhibitory functions as well as cardiac stimulation. This classification seems a valid one as far as the heart and blood vessels are concerned. Dichloroisoproterenol appears to antagonize selectively the cardiac stimulant and vasodilator effects of adrenergic stimuli, but not the vasoconstrictor actions [Moran & Perkins (55)]. On the other hand, conventional adrenergic blocking drugs such as phentolamine, phenoxybenzamine, and certain ergot alkaloids readily block the vasoconstrictor actions, but not the vasodilator or cardiac stimulant effects of adrenergic stimuli. It has been proposed that adrenergic blocking drugs be classified as either *alpha* or *beta* adrenergic antagonists, depending upon the particular type of receptor they block. The terms *alpha* and *beta* adrenergic antagonists will be used here with the understanding that this classification does not explain the nature of the receptors, but only provides a tentative heuristic framework for descriptions of drug action. Specific details of the newer aspects of adrenergic blockade are contained in following sections.

Effects of sympathomimetic amines on the heart. (a) Ventricular function and heart rate.—Although it has been adequately demonstrated that norepinephrine increases myocardial contractility [see (23)], interest still exists in the fact that norepinephrine often does not increase the cardiac output in normal subjects. It is now generally agreed that this is the result of reflex cardiac slowing caused by the rise in blood pressure produced by norepinephrine. Raab and his co-workers (56) have analyzed the effects of epinephrine and norepinephrine on the human heart, estimating changes in myocardial contractility from changes in the duration of the isometric contraction period of the cardiac cycle. They concluded that the usually observed differences in the actions of epinephrine and norepinephrine are attributable to a reflex-induced cholinergic pattern produced by norepinephrine. This reflex-induced pattern is abolished by atropine, revealing the primary adrenergic action of norepinephrine. The dependence of the pattern of the cardiovascular response to agents such as norepinephrine, epi-

nephrine, and isoproterenol upon the magnitude of the associated reflex alterations in heart rate has been nicely demonstrated by Rushmer & West (57, 58) who employed a variety of techniques in both conscious and anesthetized dogs to analyze the complex cardiovascular changes evoked by these drugs. In the absence of opposing reflex bradycardia, each of the three amines increased cardiac output and heart rate, actions in keeping with the fact that all three drugs augment myocardial contractility and automaticity. Isoproterenol increases cardiac output and heart rate even in the presence of fully intact barostatic reflexes, since the mean arterial pressure does not increase as a result of the vasodilator properties of the drug. Such effects have been observed in both dogs (57) and man [Stack *et al.* (59); Weissler *et al.* (60)].

The reflex bradycardia induced by a vasoconstrictor sympathomimetic amine has been studied by Aviado & Wnuck (61) using methoxamine, a potent vasoconstrictor drug having no stimulant action upon the heart [Goldberg *et al.* (62)]. Aviado & Wnuck thus avoided the complications which would arise from the use of an amine having a direct stimulant effect upon the automaticity of the sinoatrial node. They concluded that the bradycardia induced by methoxamine was not the result of an action on the central nervous system, since there was no effect after injection of the drug into the vertebral artery. There was also no evidence that methoxamine affected the coronary and pulmonary receptors responsible for the Bezold-Jarisch reflex, or that methoxamine had direct negative chronotropic action. Slowing of the heart by methoxamine was substantially reduced, however, by denervation of the carotid and aortic stretch receptors suggesting that the bradycardia induced by methoxamine was mediated through these structures. As Aviado & Wnuch pointed out, their experiments did not exclude an action of methoxamine on cardiac stretch receptors as a possible mechanism of the cardiac slowing. Stormorken, de Schaepdryver & de Vleeschhouwer (63) also concluded that the bradycardia produced by methoxamine was the result of reflex slowing by way of the carotid and aortic baroreceptors, but, in addition, they also found evidence for an effect of methoxamine on coronary and pulmonary receptors as well as a direct negative chronotropic action. Preziozi and his associates (64) also observed a slight negative chronotropic action of methoxamine on the perfused heart of the guinea pig.

A careful examination of the effect of mephentermine sulfate (Wyamine) has been made in dogs by Welch *et al.* (65) utilizing techniques developed by Sarnoff and associates for the evaluation of ventricular function. They found that mephentermine elevated the ventricular function curve without changing peripheral resistance and concluded that this drug is primarily a cardiac stimulant with little or no vasoconstrictor actions. They also noted that myocardial oxygen consumption increased and efficiency decreased with low filling pressures (nondilated hearts). Under conditions of high filling pressures yielding dilated hearts, on the other hand, oxygen consumption decreased and efficiency increased. These authors

aptly point out that the "oxygen-wasting" effect of sympathomimetic amines is not invariable, that amines may exert an "oxygen-conserving influence" under appropriate conditions such as cardiac dilation. They also emphasize that these two effects of mephentermine on oxygen consumption do not imply two types of metabolic effects, but that the direct metabolic effect to increase oxygen consumption may be altered by such factors as change in duration of systole, the initial filling pressure and cardiac radius.

(*b*) *Arrhythmias.*—Dresel & Nickerson (66) were unable to find evidence for a causal relationship between epinephrine-induced hyperkalemia and ventricular arrhythmias in pentobarbital-anesthetized dogs. Such a relationship had been postulated previously by O'Brien and his associates (67). The conclusion reached by Dresel & Nickerson was based upon several observations, among them a lack of quantitative correlation between the hyperkalemic response to injected epinephrine and the induction of arrhythmia, the lack of increased ability of isoproterenol to induce arrhythmias during intravenous infusion of potassium, and the lack of protection against arrhythmias by exclusion of the liver from the circulation, the latter abolishing the epinephrine-induced hyperkalemia.

Moore & Swain (68, 69) have described the properties of a new compound [U-0882 (α-phenoxy-α-dimethylaminomethyl propiophenone hydrochloride)] which sensitized the heart of the dog to ventricular fibrillation in a manner similar to that of amarine. U-0882 prolongs the refractory period of ventricular myocardium without depressing intracardiac conduction. Intravenous injections of epinephrine, norepinephrine, or isoproterenol in appropriate doses two to five minutes after the injection of U-0882 produced ventricular fibrilation. Moore & Swain postulate the initiation of a continuous conduction in the ventricles to explain the fibrillation.

(*c*)*Metabolic effects.*—Ellis (70) has reviewed the literature up to late 1958 concerning the relation of the biochemical effects of epinephrine to its effects on muscle. In addition, he presented the main lines of evidence which support his hypothesis that hexosemonophosphate may play an important role in muscle contraction, and that the actions of epinephrine on muscle are not dependent on either oxidative metabolism or on the energy produced through the anaerobic glycolytic pathway. His hypothesis places the activation of glycogenolysis in an important position.

The demonstration by Cori (71) and Rall, Wosilait & Sutherland (72) that heart muscle contains enzymes which are analogous to the phosphorylase *a* and *b* of skeletal muscle, and the observation of Ellis (73) that the phosphorylase-*a* activity of rat ventricle slices was increased by incubation with epinephrine led Hess & Haugaard (74) to evaluate the influence of epinephrine on the phosphorylase activity of myocardium. They found that addition of epinephrine to the perfusion fluid in concentrations which augmented the force of contraction of isolated rat hearts led to increased activity of phosphorylase *a* in extracts of myocardium when measured in the absence of adenylic acid, but produced no change in the activity of the total enzyme (*a* and *b*) measured in the presence of AMP. That is, epi-

nephrine stimulated the conversion of inactive phosphorylase *b* to active phosphorylase *a* simultaneously with the increased contractile activity.

Kukovetz and his associates (75) observed that other sympathomimetic amines which also have a positive inotropic effect on isolated perfused rat hearts increased phosphorylase *a* activity without changing total enzyme activity. These amines included norepinephrine, isoproterenol, epinine, synephrine, and phenylephrine. Contractile activity and enzyme activation increased in parallel with increasing doses of epinephrine and norepinephrine. On the other hand, amines such as methoxamine which had no positive inotropic effect did not activate the enzyme.

Mayer & Moran (76) compared the effects of drugs and electrical stimulation of the cardiac sympathetic nerves on the contractile force of the heart *in situ* in anesthetized open-chest dogs. Ventricular contractile force was measured directly with a strain-gauge arch, and phosphorylase activity was assayed on small samples of myocardium which were rapidly excised from the beating heart. They found, as did Hess & Haugaard (74), that epinephrine increased phosphorylase *a* activity without altering total activity of the enzyme. They also confirmed the observation of Kukovetz *et al.* (75) that only those sympathomimetic amines which increased contractile force increased the activity of phosphorylase *a*. In addition, Mayer & Moran (76) found that electrical stimulation of the cardiac sympathetic nerves augmented both ventricular contractile force and phosphorylase *a* activity, both of these responses being abolished by pretreatment of dogs with reserpine. Offering further support to the concept that contractile force augmentation and enzyme activation are parallel actions of adrenergic stimuli was the observation that the *beta* adrenergic blocking drug, DCI, antagonized both types of responses to epinephrine and nerve stimulation. However, the *alpha* adrenergic blocking agent, phenoxybenzamine, anatognized neither of these responses to epinephrine. Nonadrenergic cardiac stimulants such as ouabain, theophylline, and small doses of calcium chloride increased contractile force without augmenting phosphorylase *a* activity. These authors concluded that activation of myocardial phosphorylase is an action intimately associated with the augmentation of contractile force produced by adrenergic stimuli and that nonadrenergic cardiac stimulants do not directly influence phosphorylase activity.

The latter conclusion concerning nonadrenergic drugs is not in agreement with the observations of other workers. Hess & Haugaard (74) found that aminophylline increased both contractile force and phosphorylase *a* activity in the isolated perfused rat heart. Belford & Feinleib (77) reported activation of phosphorylase in isolated atria of guinea pigs in response to calcium chloride and K-strophanthin as well as to epinephrine. However, they observed no activation of the enzyme in hearts from intact cats which had been given K-strophanthin. The conflicting findings regarding cardiac glycosides remain to be resolved.

The question arises whether there is a common effect of epinephrine and related substances which results in increased glycogenolysis, on the one

hand, and increased muscle contraction, on the other. The demonstration by Rall & Sutherland (78) of a cyclic mononucleotide (adenosine-3′,5′-phosphoric acid; 3′,5′-AMP) in particulate fractions of several tissues such as heart, brain, liver, and skeletal muscle provides a framework for such a unitary hypothesis. These investigators found that 3′,5′-AMP promoted the formation of phosphorylase *a* from phosphorylase *b*, and that epinephrine and related amines increased the formation of this nucleotide, thus indirectly activating phosphorylase. Rall & Sutherland have suggested the possibility that 3′,5′-AMP may have other actions than activation of the kinase, i.e., it may influence muscle contraction more directly. The work in this field has been the subject of a recent review by Sutherland & Rall (79).

The new observations concerning the possible role of 3′,5′-AMP in the catechol amine-induced activation of myocardial glycogenolysis are not incompatible with the hypothesis of Ellis (70) that hexosemonophosphate accumulation is important in the action of the amines. If, in the absence of adrenergic stimuli, the reaction catalyzed by phosphorylase *a* (glycogen → glucose-1-PO_4) is a rate-limiting step in the glycogenolytic sequence, the activation of the enzyme by catechol amines should remove this limitation with some other step becoming rate limiting. If the latter is at the level of phosphofructokinase, the accumulation of hexosemonophosphate would result.

Effects of sympathomimetic amines on blood vessels.—Many papers have appeared in the past few years concerning the actions of sympathomimetic amines on blood pressure, blood flow, and on the contraction of isolated-vessel strips. Only a few of these papers will be surveyed below.

Two papers dealing with the effects of amines on the pulmonary bed are particularly noteworthy. In both, the authors carefully avoided many of the problems peculiar to this vascular bed that have beset less sophisticated investigators in the past. Borst, Berglund & McGregor (80) replaced the right ventricle of open-chest dogs with a mechanical pump and perfused each lung separately with blood. Epinephrine and norepinephrine both produced consistent, but moderate, vasoconstriction when injected into the arterial supply of one lung at a time. Aviado & Schmidt (81) measured pulmonary artery pressure in the inflow circuit to one lobe of a lung of a vagotomized, open-chest dog in which the lobe was perfused at a constant flow rate by a mechanical pump. They also measured venous outflow from a cannulated pulmonary vein of one lobe in other experiments. Their results revealed nine patterns of action of sympathomimetic drugs which varied from pulmonary constriction and increased blood flow through the lungs after administration of epinephrine or norepinephrine to vasodilation and decreased blood flow through the lungs after administration of a new drug [Compound 45-50: β-hydroxyl-β-(2,5-diethoxyphenyl) isopropylamine] having pulmonary hypotensive but systemic hypertensive properties. Isoproterenol elicited vasodilation and increased blood flow in the lungs, while methoxamine increased both pulmonary and systemic blood pressures. Although Aviado & Schmidt (81) do not discuss in detail the complex factors

contributing to the varied patterns of response, they have presented a valuable description of the pulmonary vascular effects of some 80 sympathomimetic amines, many of which are not commercially available. Aviado (82) has also critically and comprehensively reviewed the pharmacology of the pulmonary circulation.

A similar careful evaluation of the effects of 23 sympathomimetic drugs on the renal circulation of dogs was performed by Aviado, Wnuck & De Beer (83). Renal artery inflow was measured directly with a rotameter, and the responses to intraarterial injections of the amines were compared to those elicited by intravenous injections. They found four patterns of action. Type-A drugs constricted renal vessels when injected either intraarterially or intravenously (epinephrine, norepinephrine, phenylephrine, metaraminol, methoxamine, and naphazoline). Type-B drugs constricted blood vessels when injected intraarterially, but not consistently when injected intravenously (ephedrine, phenylpropanolamine, hydroxyamphetamine, and compound 45-50). Type-C amines had no important effects when injected intraarterially but increased renal blood flow when injected intravenously as a result of their hypertensive properties (amphetamine, metamphetamine, mephentermine, and others). Type-D amines increased flow when injected intraarterially but decreased flow when given intravenously as a result of their systemic vasodepressor effect (isoproterenol, isoprophenamine, and others).

Solution of the problem of the actions of sympathomimetic amines on cerebral circulation was approached by McClure & Green (84) by employing extensive ligation of the blood vessels in the necks of dogs to eliminate intra- and extracranial vascular connections. Blood flow in the carotid artery was measured with an electromagnetic flowmeter. They were unable to elicit changes in carotid artery blood flow in response to intraarterial injections of sympathomimetic amines and concluded that apparent constrictor responses obtained by other investigators using other techniques may have been caused by constriction of extracranial vessels. Whether the prolonged and extensive surgery required in this technique altered cerebral vascular reactivity has not been adequately evaluated.

The question of whether sympathomimetic amines have direct actions on coronary vessels has also engendered some controversy. Here a clear approach to the problem is complicated by extravascular effects of the drugs such as their ability to increase myocardial contractility. Berne (85) examined this problem and attempted to evaluate the extent to which the contractile actions of epinephrine and norepinephrine affected coronary flow by using dogs in which coronary blood flow and the oxygen concentration in coronary sinus blood were measured under three different conditions: (*a*) intact beating hearts, (*b*) fibrillating hearts perfused from a donor dog, and (*c*) hearts arrested with potassium and perfused from a donor dog. He concluded that the basic action of the amines on the coronaries is vasoconstriction and that secondary vasodilator responses are caused by metabolic stimulation and relative hypoxia. Denison & Green (86) found that the mean

coronary artery blood flow in dogs was increased by norepinephrine, but that the end-diastolic coronary resistance was virtually unchanged. They concluded that norepinephrine has little direct effect on arterioles of the coronary bed, and that the change in flow can be accounted for by the effect of norepinephrine on lengthening of diastole relative to the cycle length of the heart and by more rapid increase in flow to maximum values during isometric relaxation of the ventricles.

The mechanism of action of adrenergically induced vasodilatation has been studied by the Swedish workers Lundholm and Mohme-Lundholm in a series of several papers [Mohme-Lundholm (87); Lundholm (88, 89, 90)]. They present evidence favoring the view that the vasodilating effect of epinephrine is attributable to the accumulation of lactic acid as a result of the amine-induced activation of glycogenolysis in skeletal muscle. Their evidence consists, in part, of (*a*) the similarity of responses to epinephrine and lactic acid on blood flow in animals and on the relaxation of isolated artery strips, (*b*) the increase in concentration of lactic acid in blood vessels following the administration of epinephrine, and (*c*) the antagonism of the vasodilator effect of epinephrine by substances, such as copper and sodium fluoride, which block the epinephrine-induced enhancement of lactic acid production. Furchgott (91) briefly reviews some evidence which is not compatible with this hypothesis.

One final aspect of the vascular responses to sympathomimetic amines can be reviewed briefly, namely, the influence of the amines on veins. Shadle, Zukof & Diana (92) utilized a technique involving perfusion of the hind limb of a dog with constant arterial inflow and measurement of limb weight, arterial and venous pressures, and venous outflow. They found that an infusion of norepinephrine brought about initial increases in limb weight and arterial pressure which were attributed to arteriolar constriction with impounding of blood in the arteries. This was followed by a marked loss in weight and increase in small vein pressures which, they asserted, was a result of constriction of veins. From results of experiments involving the introduction of P^{32} tags into the circulation, Shadle, Zukof & Diana (92) concluded that little of the weight change was caused by a transcapillary shift of blood. Glover and co-workers (93) concluded that infusions of epinephrine and norepinephrine intraarterially in human subjects in which forearm or hand volumes were measured with plethysmographs reduced the capacity of both high-pressure (arteries) and low-pressure (veins) vessels. Eckstein & Hamilton (94) utilized the plethysmographic method with venous occlusion to obtain venous pressure-volume curves in human forearms. Infusions of epinephrine and norepinephrine increased venous pressure and decreased venous distensibility, larger changes being obtained when the arms were held in a dependent position. The *alpha* adrenergic blocking drug, phentolamine, effectively antagonized this response. The same investigators [Eckstein & Hamilton (95)] found a similar action for isoproterenol on the distensibility of forearm veins. They further demonstrated that intravenous infusions of isoproterenol reduced

atrial transmural pressure as reflected by a decreased antecubital vein pressure with no change in esophageal pressure. They concluded that isoproterenol, through a venoconstrictor effect, causes a shift of blood centrally and that the slight hyperventilation and decreased end-expiratory CO_2 tension in alveolar air were not adequate to explain the circulatory changes. In this respect Guyton and his associates (96), using a method for measuring total venous return in dogs under total spinal anesthesia, found that epinephrine increased mean circulatory filling pressure which results in increased venous return. They concluded that under normal conditions changes in cardiac output brought about by sympathomimetic amines are determined far more by increased venous return than by the heart's ability to pump blood. These five papers dealing with the effect of amines on veins leave little doubt that such amines as epinephrine and norepinephrine are capable of constricting veins, and that this venoconstriction may play an important part in the augmentation of cardiac output produced by these compounds. The finding that isoproterenol constricts veins is at variance with most of the previous studies on this compound which indicate that it is primarily a vasodilator. However, most previous work did not directly consider the action of this amine on veins. The possibility that isoproterenol may constrict veins and yet dilate arterioles, at least in skeletal muscle vascular beds, must be considered in future work.

Adrenergic Antagonists

From the work of many investigators in recent years it has become apparent that antagonism of adrenergic activity by pharmacological means can no longer be thought of in the simple terms of competitive or noncompetitive blockade. One must also consider other types of actions, such as depletion of the transmitter substance at the nerve terminals, prevention of release or synthesis of the transmitter substance at the nerve endings, and inhibition of the central outflow of sympathetic impulses. All but the last of these types of adrenergic antagonism will be considered as they relate to the cardiovascular system. The tentative classification of the various types of adrenergic antagonism used has been adopted on the basis of currently assumed modes of action of the various drugs. This classification will probably become obsolete as more information becomes available, but it is hoped that it will provide a suitable structure for the present. The classification of adrenergic receptors into *alpha* and *beta* types has been discussed above.

Drugs which antagonize adrenergic responses at the "receptors." (a) alpha-type adrenergic blockade.—Brown & Gillespie (97) have presented evidence linking the mechanism for destruction of norepinephrine and epinephrine to the functional integrity of the receptors. These investigators measured the output of "sympathin" from the splenic veins of cats in response to stimulation of the splanchnic nerves. They found that little or no sympathin could be detected in the splenic-vein blood when the nerve was stimulated at less than 10 impulses/sec., but with increasing frequencies

of stimulation from 10 to 50/sec. significant amounts of sympathin appeared. After the administration of phenoxybenzamine, and inactivation of the *alpha* receptors, the sympathin output was increased in response to nerve stimulation, and stimulation frequencies of between 1 and 30/sec. now produced large and equal outputs. Brown and his associates (98) obtained similar results using the colon and small intestine of cats. In keeping with these findings are the observations of Benfey and co-workers (99) that adrenergic blocking drugs increased the urinary excretion of epinephrine and norepinephrine, and of Millar *et al.* (100) that the concentration of catechol amines in the plasma of dogs increased after administration of phenoxybenzamine. Such data do not necessarily imply that phenoxybenzamine interferes with the enzymatic processes involved in the breakdown of catechol amines, since diminished binding of the amines to the inactivated receptors may in itself permit escape of larger amounts of intact amines into the blood stream.

These considerations provide a tentative explanation for some unpublished experiments of Moran in which it was noted that *alpha* adrenergic blocking drugs including phenoxybenzamine, phentolamine, dihydroergotamine, and chlorpromazine increased the heart rate and cardiac contractile force in open-chest dogs, but depressed isolated heart preparations. If it is assumed that these blocking drugs react only with *alpha* receptors, and since the adrenergic receptors in the heart are *beta,* not *alpha,* the increased amount of circulating catechol amines resulting from *alpha* blockade would stimulate the heart *in vivo.* In *in vitro* preparations, where there is no continual neural stimulus for the release of sympathin, no stimulant actions are observed upon addition of the *alpha*-blocking drugs. The hypothesis of Brown and his associates (97, 98) might also explain the observation of Huković (101) that stimulation of the nerve in an isolated rabbit atria-sympathetic nerve preparation produced a greater increase in heart rate after administration of phenoxybenzamine than before.

It has long been suspected that β-haloalkylamines, such as dibenamine, are metabolically altered to an active form [Nickerson *et al.* (102)]. This subject has been examined by Graham (103) who concluded that 2-haloethylamine compounds, except for fluorenyl compounds, form an ethylenimonium ion in neutral solution, and that this represents the pharmacologically active species. The chemical requirements for this type of activity have been discussed by Graham (103). Ferguson (104) also studied the adrenergic blocking properties of two haloalkylamine compounds, their ethylenimonium derivatives, and the hydrolysis products of the latter. He also considers the imonium-ring species to be the active form, but, in contrast to Graham (103), could find no relationship between the duration of the pharmacological effects and the *in vitro* life of the ethylenimonium forms.

(*b*) *beta-type adrenergic blockade.*—Powell & Slater (53) have described many of the pharmacological properties of DCI, finding that it antagonized the vasodilator actions of epinephrine. Moran & Perkins (55) observed that initial intravenous doses of DCI in dogs increased both heart

rate and force of ventricular contraction but that subsequent doses depressed the heart. Following administration of DCI, the positive inotropic response to intravenous injections of epinephrine, norepinephrine, and isoproterenol and to electrical stimulation of the cardiac sympathetic nerves was markedly reduced or abolished. The positive chronotropic response to isoproterenol in the dog was also antagonized by DCI. Specificity of antagonism was demonstrated by the failure of DCI to inhibit the cardiac stimulant effects of ouabain, calcium chloride, and theophylline. Similar results were obtained in isolated perfused rabbit hearts. Confirmation of the cardiac adrenergic antagonism by DCI has been demonstrated on isolated rabbit atria by Furchgott (91) and on the cat papillary muscle by Dresel (105). Reference has been made above to the finding of Mayer & Moran (76) that DCI also antagonizes the activation of myocardial phosphorylase induced in dogs by epinephrine and cardiac sympathetic nerve stimulation.

The work of Moran & Perkins (55) and that of Dresel (105) suggested a competitive or surmountable type of blockade by DCI on the heart. Fleming & Hawkins (106), on the basis of studies with the dog heart-lung preparation, have proposed that the antagonism by DCI should be considered as a case of competitive dualism [Ariens *et al.* (107)] and not a true example of competitive blockade. However one considers it, specificity of the antagonism lends support to the concept that the cardiac adrenergic receptors are of the *beta* type, particularly when it is recognized that conventional *alpha* adrenergic blocking drugs such as phenoxybenzamine and phentolamine lack cardiac adrenergic blocking properties [Mayer & Moran (76); Furchgott (91); Nickerson (108); and Moran & Perkins (unpublished observations)].

Drugs which antagonize adrenergic responses by preventing release or synthesis of the mediator substance, or both.—In 1957 Exley (109) published an analysis of some pharmacological effects of choline-2:6-xylyl ether bromide (compound TM-10) and concluded that it had a specific effect of either preventing the release or the synthesis of the mediator substance at the terminals of adrenergic nerves. Subsequently, similar actions were described for bretylium (N-*o*-bromobenzyl-N-ethyl-N,N-dimethyl ammonium) by Boura & Green (110), for a series of 2,6-disubstituted phenoxyethylammonium bromides by McLean and associates (111, 112), and for guanethidine [(2-[octahydro-1-azocinyl] ethyl) guanidine sulfate] by Maxwell *et al.* (113, 114). The main type of evidence for the postulation regarding the site of action of all of these drugs is the demonstration that they inhibit the response of an end organ (e.g., nictitating membrane) to electrical stimulation through either the pre- or postganglionic fibers innervating the organ, but do not inhibit axonal or ganglionic transmission or the response of the end organ to injection of epinephrine. The cardiovascular effects of these various substances are reduction in blood pressure, postural hypotension, and reduced heart rate. Maxwell *et al.* (113) feel that guanethidine is more effective as a hypotensive agent in experimental hypertensive dogs than in normotensive animals. Confirmation of the proposed mechanism of action

of these compounds must wait upon more detailed experiments as well as increased understanding of the biosynthesis and release of sympathetic neurohumoral transmitters at nerve endings.

Drugs which antagonize adrenergic responses by depleting nerve endings or effector organs of the mediator substances, or both—Much has been written about the ability of reserpine to reduce greatly the stores of catechol amines in the heart [Bertler *et al.* (115); Paasonen & Krayer (116); Waud *et al.* (117); and Lee & Shideman (118)] and blood vessels [Burn & Rand (119)]. This reduction in myocardial catechol amine content leads to a diminished responsiveness of the heart to nerve-induced adrenergic stimuli as has been shown by Trendelenberg (120) who found that the positive chronotropic response to electrical stimulation of the accelerans nerve was markedly reduced in dogs pretreated with reserpine. Similarly, pretreatment of dogs with reserpine completely abolished both the positive inotropic and chronotropic responses to stimulation of the cardiac sympathetic nerves [Mayer & Moran (76)]. Although the responses to sympathetic nerve stimulation were completely abolished by pretreatment with reserpine, the responses of the heart to injected catechol amines remained unimpaired. Lee & Shideman (118) found that isolated papillary muscles from cats pretreated with reserpine exhibited lower contractile amplitudes and catechol amine concentrations than those found in muscles taken from normal cats. Similar reductions in cardiac contractility and catechol amine concentrations were found in papillary muscles from cats subjected to chronic cardiac sympathectomies. These authors proposed that normal cardiac contractility is influenced by endogenous myocardial catechol amine stores.

As a natural consequence of the impairment by reserpine or related analogues of the sympathetic nervous system control over cardiovascular functions, hypotension, bradycardia, and reduced or abolished pressor reflexes occur [de Schaepdryver (121); Orlans *et al.* (122)]. For example, the pressor responses to carotid artery occlusion, stimulation of the splanchnic nerves and central end of the cut vagus nerve, asphyxia, and injection of TMA and nicotine are greatly reduced after pretreatment of animals with reserpine. The depression of the pressor responses has been shown to be accompanied by a reduction in catechol amine content of the adrenal glands, heart, liver, and spleen [de Schaepdryver (121)]. Further evidence that the reduction in sympathetic activity is a result of depletion of peripheral stores of norepinephrine by reserpine-like substances rather than to an effect on the central nervous system has been provided by Orlans *et al.* (122) who observed that all of the peripheral actions of such drugs were evident after injection of syrosingopine, a semisynthetic reserpine alkaloid, in the absence of signs of a tranquilizing effect or depletion of serotonin or catechol amines from stores in the brain.

The acute release of catechol amines by reserpine is accompanied by a positive chronotropic and inotropic action in the dog heart-lung preparation [Paasonen & Krayer (116); Krayer & Fuentes (123)]. The positive chron-

otropic effect of reserpine has been shown to be accompanied by a depletion of myocardial catechol amine stores [Paasonen & Krayer (116)]. Plasma levels of catechol amines did not increase while the myocardial stores were being depleted, but the release may have been sufficiently slow so that the amines were destroyed as rapidly as they were released. Once the myocardial catechol amine stores were depleted by pretreatment of a dog with reserpine, further administration of reserpine when a heart-lung preparation was made did not evoke a positive chronotropic response.

Administration of reserpine to intact animals also produces evidence of sympathetic stimulation including a positive inotropic action [Cotten & Moran (unpublished observations)] and a rise in blood pressure [Domino & Rech (124); Maxwell *et al.* (125); Horita (126)]. The pressor response develops within five to 20 minutes after injection of 1 mg/kg. of reserpine in unanesthetized dogs [Domino & Rech (124)]. In these experiments ganglionic blockade enhanced the response, pentobarbital reduced it, phenoxybenzamine abolished it, and bilateral adrenalectomy did not prevent it. Maxwell *et al.* (125) also noted that intravenous injection of reserpine produced pressor responses in anesthetized dogs that had received large doses of ganglionic blocking drugs. The response was reduced by phentolamine. Lysergic acid diethylamide or spinal cord section at C-1 or C-2 had no effect on the response. Horita (126) made similar observations, finding that intravenous injections of reserpine into anesthetized dogs produced pressor responses if the dogs had been pretreated with cocaine. This response was antagonized by adrenergic blocking agents but not by 2-brom-lysergic acid diethylamide. The response was decreased but not abolished by adrenalectomy. The most obvious mechanism for this pressor response would appear to be an elevation of free catechol amines in plasma and tissue as a result of the acute release of the bound amines, but none of these papers report determinations of plasma levels of amines. However, Muscholl & Vogt (127) found that intravenous injections of reserpine in rabbits raised adrenaline concentration in plasma by 54 to over 1000 per cent. Norepinephrine concentration was too low to determine any change.

Drugs Which Produce Cardiovascular Sympathomimetic Effects Through Indirect Actions

Brief mention should be made of an increasingly important aspect of drug action: the indirect production of effects through the release of endogenous substances. Evidence is accumulating that cardiovascular responses to a number of drugs may be mediated, at least in part, through the release of catechol amines, either from the adrenal medulla or from tissue stores. Burn & Rand (119) have proposed that the blood vessels contain stores of norepinephrine and that certain drugs can cause either a temporary or a prolonged release of the amine. They also proposed that the stores, once depleted, can be replenished by exposure to norepinephrine. They have found, for instance, that the vasopressor effect in spinal cats,

the vasoconstrictor effect in perfused dog hind limbs and rabbit ears, and the contraction of isolated strips of rabbit aorta produced by tyramine and other noncatechol amines are greatly reduced or abolished by pretreatment of the animals with reserpine. The responses to catechol amines, on the other hand, are potentiated. The action of tyramine and related amines can be restored in the reserpinized preparation by infusion of norepinephrine.

Burn & Rand (128) have also found that the vasoconstriction produced by nicotine and acetylcholine (in the presence of atropine) in the perfused rabbit ear is abolished by reserpine. Reserpine also reduces the amount of a norepinephrine-like substance in the skin of the rabbit ear as well as apparently diminishing the chromaffin granules in the skin.

Richardson & Woods (129) have demonstrated a release of norepinephrine from the isolated perfused rabbit heart in response to acetylcholine when atropine is present in the perfusion fluid. The release of the catechol amine corresponded in time with the increased contractile force resulting from administration of acetylcholine.

Ganglionic stimulants, such as tetramethylammonium (TMA), nicotine, and acetylcholine (in the presence of atropine), produced increased force of contraction of papillary muscles from cats [Lee & Shideman (130)]. This effect was blocked by ganglionic blocking drugs, compound TM-10, and DCI. The effect of epinephrine on the papillary muscle, however, was not blocked either by ganglionic blocking drugs or compound TM-10. The muscle became refractory to repeated administration of the ganglionic stimulant drugs. In papillary muscles from reserpine-treated cats, in which the catechol amine concentration was reduced, TMA and nicotine had little or no effect, but the muscle was fully responsive to epinephrine or norepinephrine. Histological examination of the papillary muscles revealed no ganglion cells. Lee & Shideman concluded that the cardiac stimulation is either mediated through postganglionic sympathetic nerve endings in the heart or through elements that can be classified pharmacologically as ganglia but which do not meet the morphological criteria for such a classification. At any rate, these drugs appear to act through release of catechol amines from the stores in the myocardium. Lee & Shideman (131) subsequently conducted a detailed analysis of the structure-activity relationships of quaternary ammonium compounds regarding the actions on the heart. They found that all of a large series of quaternary ammonium compounds had positive inotropic actions, although in some compounds this action was masked by prominent muscarinic activity. Presumably, these agents act through the same mechanism utilized by acetylcholine, TMA, and nicotine.

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RENAL PHARMACOLOGY[1,2]

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In the preparation of the first review of an annual series, the authors have a unique opportunity. A consideration of only material published in the last year or two would hardly suffice as an adequate background for the reviews to follow; it therefore seems essential to include a wider selection of work to define more adequately the present status of the field. At the same time, freed of the necessity of providing a more or less complete bibliography of material related to the assigned subject for the immediately preceding period, the authors have more freedom in the choice of work to be included and in the presentation of selected areas of study.

Pharmacological interest in the kidney is directed largely along two lines —the effect of drugs on the transport mechanisms of the renal tubules and the effect of drugs upon the renal circulation and glomerular activity. This review will deal primarily with the renal transport mechanisms for electrolytes and water and the action upon them of several groups of diuretics. Limitations of space have required that several subjects of considerable current interest (e.g., the actions of hormones on renal function, uricosuric agents) be omitted except where directly pertinent to other material.

The regulation of salt and water excretion is, from the point of view of the amounts of substance involved, far and away the most important function of the renal tubules. In addition, it has been the function most effectively subjected to pharmacologic modification for therapeutic purposes. It is pertinent to a consideration of substances which affect these transport mechanisms to review current concepts of the site magnitude, and nature of the processes involved.

Functional Organization of the Kidney

Proximal tubule.—Recent micropuncture studies have, in general, confirmed earlier inferences (1, 2, 3) concerning the extent of sodium, chloride, and water reabsorption in the proximal convoluted tubule and concerning the primacy of active sodium transport in this process. In confirmation of the earlier studies of Walker *et al.* (2), Lassiter, Gottschalk & Mylle (4) have found that in the rat some 65 per cent of the glomerular filtrate is reabsorbed in the convoluted portion of the proximal tubule. Extrapolation to the end of the proximal tubule yields a figure approximating the 80

[1] The survey of the literature pertaining to this review was concluded in July, 1960.

[2] Abbreviations used in this chapter include PAH (*p*-aminohippurate).

per cent estimated earlier (2). The finding that in amphibia (5) and in mammals under the influence of antidiuretic hormone (2, 6, 7) the fluid in the proximal tubule is always isotonic with plasma has been extended to the rat in water diuresis (6, 7). This suggests, as has long been assumed, that the proximal tubule has a high water permeability and that this high permeability is not influenced by antidiuretic hormone. This permeability has been measured directly in the amphibian, *Necturus,* by Whittembury and his associates (8), who have, in addition, demonstrated the entirely passive nature of water movement in this segment, by showing that net water movement out of the tubule lumen is entirely dependent upon net solute movement (9). (In fact, under the conditions of their study which involved instillation of solutions of defined composition into the tubule, it was possible to produce net solute movement, and hence net water movement, into the lumen, in the direction counter to the normal net flux of solute and water.)

The early view emphasized by Bayliss (10) that the movement of fluid and solute out of the proximal tubule might result from the osmotic force provided by the plasma protein in peritubular capillaries has undergone spirited, if short-lived, revival in the last few years (11, 12). Evidence opposed to this possibility has developed rapidly. Whittembury and his coworkers (8) have shown that the inclusion of plasma albumin at concentrations exceeding that in peritubular blood has a negligible effect on the movement of solute and water out of the tubule of *Necturus.* Furthermore, from their measurements of the permeability of the proximal tubule to water, they have been able to calculate the rate of water movement which might be produced by an osmotic pressure of the magnitude attributable to the plasma proteins, and this proves to be a negligible fraction of that actually observed (8). On the other hand, there is abundant direct evidence supporting earlier inferences (13, 14) that the movement of sodium out of the proximal tubule involves transport against a gradient of electrochemical potential and is thus, by definition, "active." The lumen has been found by Solomon (15) in the rat and by Giebisch (16) and Schatzmann *et al.* (17) in *Necturus* to be electrically negative with respect to the interstitial space of the kidney by approximately 20 mv. The movement of sodium from lumen to blood is thus against an electrochemical gradient, even if, as is usually the case, there is virtually no difference between the chemical sodium concentration of the fluid in the lumen and that in the blood. However, when the tubule contents include a considerable amount of solute to which the tubules are impermeable (e.g., mannitol) the reabsorption of sodium reduces the concentration of sodium in the lumen producing a gradient of chemical concentration as well as electrical potential. This has been shown to be the case both in the rat (18) and in *Necturus* (9). It should be noted, however, that the concentration gradient against which sodium can be transported is limited, so that in *Necturus,* in which the studies are more complete, net outward movement ceases when the con-

centration of sodium in the tubule has been reduced to about 60 per cent of that in the plasma (9). This is presumably attributable to the high passive permeability of the tubule to sodium, the diffusion leak of sodium along its electrochemical potential gradient just balancing the capacity of the tubule to transport it outward.

As for the movement of chloride, the direction of the electrical gradient and the high permeability of the proximal tubule to this anion are sufficient to account for its reabsorption from the tubule as a passive process (19, 20). However, the data of Giebisch (20) indicate that the ratio of outflux to influx as measured with tracers is not as large as would be expected for a simple process of diffusion as predicted from the flux ratio equation of Ussing (21). This type of departure from the flux ratio equation is usually taken to indicate the involvement of exchange diffusion (22, 23), and the latter phenomenon is usually considered to suggest the participation of a carrier in the ion movement. It may be, therefore, that in its movement across the tubule cell, chloride may combine with some cell component so that its passage is not entirely in the free state, even though it is energetically downhill.

As in the case of such transport processes generally, virtually nothing is known about the details of the active process by which sodium is moved from lumen to interstitial space. In the elucidation of such mechanisms, their intimate nature, energy sources and substrates, lies the key to the rational development of agents for their modification. To date, the development of drugs modifying renal electrolyte transport has been entirely empirical.

Loop of Henle.—Considerable recent interest has centered upon the loop of Henle because of its involvement in the formation of hypertonic urine. Although it seems clear that, as first proposed by Wirz, Hargitay & Kuhn (24), the loop acts as a countercurrent multiplier and that the primary active process involved is transport of sodium from lumen to medullary interstitial fluid, considerable question remains as to the details of the process. It is clear that in animals excreting hypertonic urine the fluid flowing through the loop of Henle becomes concentrated as it flows down the descending limb, reaching, at the tip of the loop, a concentration not measurably different from that of the final urine (7, 24) and then becoming diluted even more rapidly as it flows back up the ascending limb to the cortex (6, 7). It seems most likely that the rising concentration in the descending limb is due primarily to loss of water to the hypertonic surroundings (24, 25), although, at present, it can not be rigorously excluded that there is not some uptake of sodium chloride in addition to the net gain of urea which has been shown to occur (4). Thus, the descending limb appears, at least in antidiuresis, to share the permeability properties of the proximal tubule. Whether, in the absence of antidiuretic hormone, it may become considerably less permeable to water as does the distal tubule remains to be determined. The dilution which occurs in the ascending limb is

clearly a result of the removal of sodium and chloride (not to the addition of water) since the fluid arrives in the distal tubule, not only dilute (6, 7) but reduced in volume below that at which it entered the loop (4). The characteristics of the process that moves sodium chloride out of the ascending limb are unknown. It is generally assumed to involve the active transport of sodium and the secondary movement of chloride, but this is based on analogy rather than evidence. The countercurrent multiplier effect, which provides a high salt concentration within the lumen at the point at which the interstitial concentration is also high and a lower surrounding concentration when the internal concentration has been reduced, keeps small the concentration gradient against which sodium chloride is transported so that it is not known there is a limitation similar to that found in the proximal tubule. It is important to note that the salt transported out of the ascending limb plays a central role in both the process by which the urine is concentrated and that by which it is diluted. It is the salt moved from the loop and deposited in medullary interstitium which maintains the high osmotic pressure around the collecting ducts (26). At the same time a very considerable dilution occurs which, if maintained, is sufficient to account for a major fraction of the solute-free water excreted in water diuresis.

Distal convoluted tubule.—The generally held view of the role of the distal convoluted tubule in water and electrolyte excretion has been somewhat blurred by the demonstration of considerable transport activity in the collecting ducts in the hamster (27, 28). As a result, there is some uncertainty concerning the extent to which activities formerly attributed to the "distal tubule" really are located in the distal convoluted tubule or the collecting system. In any case, it appears that both segments share one important property, namely, a low permeability to water in the absence of pituitary antidiuretic hormore (vasopressin) and a high permeability in its presence (6, 7, 29). In the presence of antidiuretic hormone, the excess of water over solute present in the contents of the first part of the distal convoluted tubule is, at normal rates of flow, more or less rapidly dissipated (6, 7) as it progresses down the tubule, and the fluid remains close to osmotic equilibrium with the peritubular fluid thereafter. In water diuresis, on the other hand, the permeability of the distal system is sufficiently low to maintain the dilute character of the urine despite the much higher osmotic pressure of the peritubular fluids.

Certain differences in sodium transport are apparent between proximal convoluted tubule and distal system. The most striking of these is the ability of the distal system to reduce the salt concentration virtually to zero as compared with the limited concentration gradient which can be established in the proximal tubule. In addition, a fraction of the removal of sodium in the distal system involves replacement of the sodium with potassium, hydrogen, and ammonium ions and is thus not accompanied by an equivalent abstraction of anion (30, 31). On the other hand, it is not certain to what extent these differences are attributable specifically to dif-

ferences in the sodium transport mechanism itself or to differences in such parameters as the passive permeability of the tubule cells to sodium, potassium, chloride, and water, and to the modification which has already occurred in the composition of the fluid in the tubule lumen by the time it reaches the more distal portions of the nephron.

Measurements of the electrical potential gradient across the distal convoluted tubule are less numerous than those which have been made in proximal convolutions. Contrary to the early findings of Wilbrandt (32), all recent determinations have indicated that the lumen is negative with respect to the interstitial fluid (15, 16). In addition, the magnitude of the electrical potential gradient has been greater in distal than in proximal tubules. Thus the direction of the potential is favorable to the loss of chloride from the lumen by diffusion and is sufficient to produce an appreciable gradient of chemical concentration. However, the production of the frequently observed urinary concentrations one-hundredth that in plasma would require potential gradients of the order of 120 mv. or more. This is approximately twice as great as any which have been measured to date. It is clear that the conditions under which the few distal tubule potentials have been measured are not necessarily those under which maximum chloride reabsorption occurs; nevertheless, it can not be accepted as established that chloride reabsorption in this segment does not require an active transport mechanism.

Collecting duct.—The recent studies of Ullrich and his associates (27, 28) and of Hierholzer (33) have established that, in the hamster, many of the electrolyte transport processes attributed to the "distal tubule" are present in the collecting ducts as well as, presumably, the distal convoluted tubule. The extent to which a similar situation exists in other species remains to be determined, although the studies of Jaenike & Berliner (34) suggest that there may be removal of sodium and chloride and addition of potassium in this segment in the dog. The reviewers do not share the view of Ullrich that the salt transported out of the collecting duct contributes to the process of forming hypertonic urine, but they believe that it must contribute to the over-all regulation of sodium excretion as well as that of potassium, acid, and ammonia and to the dilution of the urine in water diuresis. A contribution of the collecting system to the dilution of urine, at least in rodents, is apparent from the studies of Wirz (6) and Gottschalk (7).

The studies involving fluid collection from the collecting ducts of the hamster did not include measurements of chloride concentration. Therefore, although it is established that sodium reabsorption occurs in this segment, it is not clear whether this is entirely attributable to the demonstrated cation exchanges or whether there is also some removal of chloride. The studies of Jaenike & Berliner (34) suggest that chloride is reabsorbed from the collecting system of the dog kidney. If the latter process occurs, it will be difficult, indeed, to explain it entirely as the passive result of an electrical potential gradient because (*a*) the very high chloride concentra-

tion of medullary interstitium would add to the chemical gradient and thus impose the necessity of some 40 to 50 mv. beyond that required for similar movement in the distal convoluted tubule (see above) and (*b*) the large caliber of the collecting ducts and the short distance to the pelvis would make it difficult to maintain such a high potential in the face of the considerable shunt represented by the conductance of the fluid in the collecting ducts. These considerations again emphasize the necessity of retaining the possibility of active chloride transport in any view of renal transport processes and their modification by drugs.

Potassium.—The specific contribution of various tubule segments to the modification of potassium excretion is by no means as clear as is the case for sodium, chloride, and water. Considerable evidence has accumulated in support of the view that a major part of the filtered potassium is reabsorbed before most of the potassium destined for excretion is secreted into the tubule fluid (35, 36, 37). It is clear that all or virtually all of the filtered potassium can be reabsorbed since urine almost free of potassium can be produced. Unless it should be unexpectedly shown that there is at times complete reversal of the electrical gradient, the excretion of a urine with a potassium concentration lower than that in plasma requires active transport of the potassium out of the lumen. There is, in addition, considerable experimental support for the view that, even under conditions in which large amounts of potassium are excreted, all or very nearly all of the filtered potassium is reabsorbed and that which appears in the urine is derived from the secretory process (36). However, the site of the reabsorption is uncertain. Earlier micropuncture studies suggesting a lowering of potassium concentration in the proximal tubule in both *Necturus* and rat (38, 39) have not been confirmed in more recent work (40), and the latest observations of Oken & Solomon (41) indicate that in *Necturus* the concentration in the lumen of the proximal tubule is higher than in plasma and close to the value required by the electrical potential with potassium at electrochemical equilibrium—that is, with the electrical gradient equal and opposite to the chemical concentration gradient. If the same is true in the mammal, this would require that the active reabsorptive process be located at a more distal site. However, some caution is required in this interpretation since there have been no studies of the gross behavior of potassium excretion in *Necturus* to indicate that it is similar to that in mammals. In stop-flow studies in the dog, the lowest potassium concentrations are found in those "early distal" samples which also contain the lowest sodium concentrations (42). This has been interpreted by Sullivan *et al.* (42) as indicating that these samples delineate the site of maximum potassium reabsorption. This conclusion is certainly not warranted by the evidence for reasons which have previously been presented *in extenso* (43, 44). In essence, one can not distinguish whether the potassium concentration of these samples is low because of potassium reabsorption at that site or because, having been rendered virtually sodium-free, these samples can not take up potassium in ex-

change for sodium as they subsequently flow past the site of potassium secretion. It may be concluded that the site of potassium reabsorption is not distal to the locus proposed by Sullivan *et al.* but could be situated there or at any point proximal to it (44). These considerations become important in the interpretation of stop-flow studies of pharmacologic agents (see below).

Some confusion concerning the role of the distal convoluted tubule versus that of the collecting duct in the secretion of potassium has arisen from the observations of Hilger *et al.* (45) suggesting potassium secretion in the collecting system in the hamster. Although later, more extensive observations by the same investigators (27) failed to establish the occurrence of such secretion under the conditions of study, Hierholzer (33) has demonstrated that potassium secretion does take place in the collecting ducts of potassium-loaded hamsters after acetazoleamide injection. The experiments of Jaenike & Berliner (34) suggest that there is potassium secretion in the collecting system of the dog as well, but indicate that the process extends well back into the distal tubules. The latter conclusion can be reached a priori, in any case, because the amounts of potassium that can be secreted exceed considerably the amount of potassium in the very low blood flow to the renal medulla (44).

The mechanism of potassium secretion clearly involves exchange for sodium ion. This conclusion has received additional support in the last few years (36, 46). It has not been established that the process is active as far as the movement of potassium is concerned, i.e., the electrical gradient with lumen negative to interstitial fluid may be sufficient to account for the accumulation of potassium in the urine. However, as in the case of chloride, the electrical potential required to account for the observed concentration is greater than any which has yet been shown to exist. Whether or not the potassium accumulation is attributed to an active process may prove to be a semantic question in view of the indications that in other analogous situations the active transport of sodium, presumed to be the initiating process in establishment of the electrical potential gradient, involves specific exchange of sodium for potassium (47, 48).

Bicarbonate, urinary acidification, and ammonia excretion.—Measurements of the pH of fluid collected from the proximal convoluted tubule of the rat (49) have confirmed the inferences of Walker *et al.* (2) that the bicarbonate concentration of fluid in the proximal tubule of this species is lower than that in plasma. Although it has generally been accepted in recent years that bicarbonate reabsorption in the proximal tubule, now indicated as being of the order of 90 per cent or more of the filtered bicarbonate, is attributable to exchange of hydrogen ion for reabsorbed sodium, some of the consequences of this assumption have been pointed out for the first time by Walser & Mudge (50). The hypothesis implies the combination of the secreted hydrogen ion with bicarbonate to form carbonic acid which in turn is split to CO_2 and water, the CO_2 escaping by diffusion. This requires, then, that carbonic acid be broken down at a rate equal to that at which

bicarbonate is reabsorbed. In order to achieve this rate, (*a*) there must be accumulation of carbonic acid to a concentration providing the indicated rate of breakdown by the uncatalyzed reaction $H_2CO_3 \rightleftharpoons CO_2 + H_2O$ (the required carbonic acid concentration would produce a pH approximately one unit lower than that achieved when the above reaction has come to equilibrium) or (*b*) the reaction in the lumen must be catalyzed (one would presume by carbonic anhydrase on the cell surfaces since it is clearly not present in the urine). The technique used for the pH measurements which have so far been made does not exclude the possibility of a pH *in situ* lower than that observed after isolation of the sample for analysis (49, 51), but it seems rather unlikely that this is the case. The remaining alternatives would then be (*a*) a complete revision of our views of the mechanism of bicarbonate reabsorption to one which does not involve formation of carbonic acid in the lumen or (*b*) a new role for carbonic anhydrase in the process—i.e., the catalysis of carbonic acid breakdown in the lumen.

The localization of urinary acidification and ammonia excretion in the distal segments of the mammalian kidney, assumed since the observations of Montgomery & Pierce (51) and Walker (52) in amphibia, has been confirmed on the basis of stop-flow studies (42, 53, 54). An appreciable part of the ultimate lowering of pH apparently may occur in the collecting system in the rat (49) and the hamster (55). Secretion of ammonia also occurs in the collecting ducts of the hamster (28), but it is unlikely that this is the major site of secretion since the supply of precursors of ammonia to this segment is so limited by the low level of medullary blood flow (44). Furthermore, in the rat, the rate of excretion of ammonia is very well correlated with adaptive changes in the glutaminase activity of the cortex while these adaptive changes are absent in medulla and papilla (56, 57).

Transport and excretion of organic acids and bases.—The renal transport mechanism which has probably been more extensively studied than any other is that responsible for the secretion into the urine of a wide variety of organic acids, most familiarly *p*-aminohippurate (PAH), diodrast, and phenol red. The similar but separate mechanism which transports a number of organic bases into the urine has been more difficult to study because of the generally high physiologic activity of most of its substrates. Taggart (58) and Sperber (59) have reviewed the process by which organic anions are transported while Peters (60) has reviewed the transport of organic bases.

The most important development in this field has been the clarification of the interrelationship between active transport and passive diffusion in the excretion of a number of organic compounds in both the acidic and basic series. It has been known for some years that the rate of excretion of a number of weak acids and bases is dependent upon the pH of the urine, the excretion of weak acids increasing, and the excretion of bases decreasing as urine pH increases (61). This has been attributed to the fact that cell

membranes are generally highly permeable to the lipid-soluble un-ionized species but very much less so to the ions (62), a phenomenon which has been designated as nonionic diffusion (63). Since permeation of the tubule is limited almost exclusively to the un-ionized form, diffusion equilibrium is reached only when the un-ionized form is present in equal concentration on both sides of the membrane; the total concentration of the weak acid or base required to provide a given concentration of the undissociated species is, of course, a fuction of pH. Although there can be little question of the general validity of this interpretation, it has become apparent that in the excretion of at least a number of these weak acids and bases more is involved than non-ionic diffusion alone. It was found by Gutman *et al.* (64) and by Schachter & Manis (65) that the excretion of salicylate, a substance showing the relationship between urine pH and excretion typical of nonionic diffusion, was markedly depressed by probenecid, a well-known inhibitor of the active transport mechanism responsible for the secretion of PAH and other organic anions. This phenomenon has been explored extensively by Weiner, Washington & Mudge (66) who present convincing evidence that salicylate is secreted in the proximal tubule by the same mechanism that effects the transport of PAH and that it is subsequently variably reabsorbed by passive nonionic diffusion from the distal nephron at a rate inversely related to the pH of the urine. They show that salicylate secretion is inhibited not only by probenecid but by PAH, acetazoleamide, and benzmalecene as well. The same group has explored this type of phenomenon further and has found a wide variety of organic acids to exhibit similar behavior (67). Probenecid is perhaps the most striking example since it has long been considered the typical "refractory substrate" inhibitor—that is, a substance with a high affinity for the transport mechanism but not itself subject to transport (68). Although it has an extremely low clearance under usual conditions of acid or neutral urine, probenecid is excreted at rates far in excess of that at which it is filtered when the urine is rendered alkaline (67); furthermore, its excretion is depressed by PAH. As Weiner *et al.* point out (67) these findings introduce new complications into the already confusing question as to the structural requirements for active secretion of organic acids. They further indicate that if such secretion is to result in a high rate of urinary excretion, the organic acid must have a low lipid solubility or the urine a very high pH.

Meanwhile it has become apparent that similar considerations apply to the excretion of weak bases. Volle, Green & Peters have found that quinine, quinacrine, and mecamylamine inhibit the secretion of *N*-methylnicotinamide in the chicken (69), although the first three substances generally have rather low clearances and are among those which, in the dog, have clearances which vary greatly with urine pH (61, 70, 71). Torretti *et al.* report that in the dog there is evidence of active secretion of quinine and that nonionic diffusion is concerned chiefly with its loss from rather than uptake

into the urine (72). It thus appears that among the bases as well as the acids the structural requirements for active transport are extremely nonspecific.

Chlorothiazide and Related Compounds

Chlorothiazide, synthesized by Novello & Sprague (73) and introduced as a diuretic by Beyer and his associates (74), has become the prototype of an extensive and still expanding series of heterocyclic sulfonamides with diuretic properties. These compounds have considerable pharmacologic and therapeutic interest not only because of their intrinsic activity but also because they are highly effective on oral administration. The original compound was the by-product of an attempt to produce a carbonic anhydrase inhibitor of greater potency by the inclusion of two functional sulfonamide groups. The benzothiadiazine compound showed an unexpected capacity to produce enhancement of chloride excretion in addition to those actions usually associated with carbonic anhydrase inhibition. Although the capacity of chlorothiazide to inhibit carbonic anhydrase was at first considered the basis of its chloruretic action (74), it has become increasingly clear that these are separate properties. Most of the more recently introduced compounds of more or less related chemical structure have considerably enhanced chloruretic activity but diminished potency as carbonic anhydrase inhibitors.[3] It has, in fact, been reported by Logemann *et al.* (75) that several similar compounds substituted on the sulfonamide nitrogen, and therefore devoid of carbonic anhydrase inhibitory activity, retain the capacity to produce diuresis. The relationship between structure and activity has not been established; nearly all of those which have been so far introduced are derivatives of benzothiadiazine, but there are exceptions (76) which indicate that this ring structure is not itself essential.

Administered in fairly large doses, chlorothiazide increases the excretion of sodium, chloride, potassium, and bicarbonate (74, 77). Maximal effects with approximately 10 per cent of the filtered sodium and chloride excreted in the urine are produced in the dog at doses of approximately 10 mg./kg. (77). When maximally effective doses of a mercurial diuretic are superimposed upon the chlorothiazide diuresis, there is a marked further increase in sodium and chloride excretion. When the order of drug administration is reversed, similar additive effects are produced. From this Pitts and his associates (77) infer that the effects are independently exerted on separate systems yielding energy for the mechanism which effects the tubular reabsorption of sodium.

On the other hand, there was no further increase in the excretion of potassium and bicarbonate when chloromerodrin was administered to a dog undergoing chlorothiazide diuresis, but the excretion of potassium is mark-

[3] Because of space limitations and because there have been no major developments in the study of the renal effects of carbonic anhydrase inhibitors since the subject was last reviewed (82), this group of agents is not dealt with in this review.

edly inhibited (77) indicating that the enhanced potassium excretion is attributable to enhanced secretion rather than inhibited reabsorption (78). The effects of chlorothiazide on the excretion of bicarbonate and potassium are entirely characteristic of the effects of carbonic anhydrase inhibitors, and there was no enhancement of these effects when chlorothiazide was given to animals pretreated with the more powerful carbonic anhydrase inhibitor, acetazoleamide. That these effects are, indeed, attributable to the carbonic anhydrase inhibition produced is further indicated by the facts that (*a*) smaller doses of chlorothiazide may produce the enhanced excretion of sodium chloride without significant effect on urine pH or potassium excretion (74), (*b*) derivatives of chlorothiazide possessing higher ratios of chloruretic to carbonic anhydrase inhibitory activity yield high rates of sodium and chloride excretion without modifying bicarbonate excretion and with only those increases in potassium excretion to be expected, for reasons discussed below, from any diuretic agent (79, 80).

The major diuretic effects of chlorothiazide are not modified by acidosis or alkalosis (74). This is a significant departure from the situation with organic mercurials, the effects of which are markedly suppressed by administration of alkali (see below). On the other hand, failure of metabolic acidosis to suppress the action of chlorothiazide is a departure from the behavior of drugs owing their action entirely to the capacity to inhibit carbonic anhydrase (81, 82), although the absence of enhanced bicarbonate excretion when chlorothiazide was given to acidotic animals (74) may be interpreted as suppression of this facet of chlorothiazide action.

Since the production of potassium depletion has been one of the few limitations in the therapeutic use of chlorothiazide and its congeners, a more detailed consideration of the effects of these agents on renal tubular transport and excretion of potassium is warranted. That carbonic anhydrase inhibitors increase potassium excretion and that this effect is specifically attributable to enhanced secretion of potassium is well established (54, 78). Since significant renal carbonic anhydrase inhibition is produced with most therapeutic doses of chlorothiazide, marked increases of potassium excretion are to be expected. However, since many of the more potent derivatives are diuretic at doses which yield negligible carbonic anhydrase inhibition, the effect on potassium excretion is predictably and demonstrably much smaller (81, 82). However, all diuretics share the property of increasing the excretion of potassium, even though they have no specific effect on potassium transport or despite the fact that, as is the case with mercurial diuretics, they may have the specific capacity to inhibit the secretion of potassium by the renal tubules. This property derives from the fact that, to the extent that they are effective, they enhance the delivery of sodium salts to the site of sodium-potassium exchange in the more distal portions of the nephron and thus permit the more efficient utilization of the capacity to secrete potassium (83, 84). Although stop-flow studies of the effects of chlorothiazide have been interpreted as indicating inhibition of potassium reabsorption (85), these conclusions are

not valid since the method does not distinguish diminished reabsorption from enhanced secretion as has been previously pointed out (44).

A further factor tending to potassium depletion when benzothiazide diuretics are used relates to the increased capacity to reabsorb sodium and secrete potassium, which is a feature of those individuals with disorders leading to the formation of edema and in individuals depleted of sodium. Many of those to whom benzothiazide diuretics are administered fall in one or the other of these categories. It is to be expected that in such individuals loss of potassium will be considerably greater than when a diuretic is administered for the first time to a normal individual. Indeed, a progressive increase in potassium excretion can be shown to occur with time in individuals given regular doses of diuretics (86). The data of Edmonds & Wilson (87) strongly support the interpretation that such enhancement of potassium excretion results from increased secretion of aldosterone, and Edmonds (88) and Liddle (89) have shown that antagonists of aldosterone increase the excretion of sodium and diminish that of potassium when benzothiodiazine diuretics (hydroflumethiazide) are administered. In Edmonds' study (88) this was particularly marked in those patients previously showing the smallest excretion of sodium and the largest excretion of potassium in response to the diuretics.

In view of these considerations and the fact that no evidence has been adduced to indicate that benzothiazide diuretics have more than two specific actions—that on carbonic anhydrase and that responsible for their chloruretic effects, one may anticipate that once the chloruretic potency is great enough to yield maximum chloruresis without carbonic anhydrase inhibition, all of the drugs will have similar diuretic properties. One may, therefore, view with skepticism claims that among such potent chloruretic agents there are significant differences in their effects on potassium excretion (90, 91, 92).

Inferences concerning the locus of action of these diuretics in the renal tubule have been based on the application of two techniques, and unfortunately, the results obtained with the two procedures are almost diametrically opposed. Kessler and his associates (93) and Vander *et al.* (85) from studies using the stop-flow technique in dogs have concluded that the major effects of chlorothiazide on the reabsorption of sodium and chloride are exerted in the proximal convoluted tubule. (There is an additional distal tubule effect on pH, ammonia, and potassium excretion characteristic of carbonic anhydrase inhibitors.) On the other hand, a number of investigators have studied the effect of one or another diuretic of this group on the excretion of sodium, chloride, and water in man (94, 95, 96) and in dogs (97) undergoing water diuresis. The data obtained, particularly in the dog, strongly support the view that the major effect is upon the reabsorption of sodium and chloride in the distal system where sodium reabsorption leads, in water diuresis, to the production of dilute urine. The latter workers have found that in maximum water diuresis, the administration of thiodiazine

diuretics produces an increase of sodium and chloride excretion with essentially no change in urine flow. This is the behavior to be expected if the volume and salt content of fluid delivered to the site of dilution remains unchanged, but there is inhibition of the process of salt removal leaving water behind, which normally yields a dilute urine. These observations seem clearly to point to a site of action in the distal portions of the nephron—that is, the ascending limb of Henle's loop or beyond. The discrepancy seems to the reviewers to indicate the weakness of the stop-flow technique as a method for localizing activities in the renal tubule, particularly with regard to the more proximal of two or more processes effecting the transtubular movements of a substance. In the case of chlorothiazide, it would appear that the changes in distal tubule function produced by the drug are not sufficient to prevent the virtually complete removal of sodium during the prolonged period of stasis in stop-flow, but are sufficient to impair the removal of sodium as fluid from higher in the nephron passes this segment when flow is re-established.

A finding apparently related, at least in part, to the capacity of thiodiazine diuretics to impair urinary dilution is the interesting observation of Crawford & Kennedy (98, 99) that their administration to patients with diabetes insipidus leads to a marked reduction in urine flow and an increase in urine concentration. The increased concentration which occurs when the drug is first administered is quite in accord with its effects in normal animals in water diuresis (97). The subsequent decrease in urine flow is not so easily explained, but must be attributable to a decrease in the fraction of the glomerular filtrate escaping reabsorption in the proximal tubule. It would appear from studies of Earley & Orloff (86) that once a sodium deficit has been produced, the reduced urine volume and increased urine concentration can be maintained without further administration of drug, so long as the sodium deficit is maintained by a rigidly restricted salt intake; the changes in excretion are promptly reversed when the losses of salt have been restored. These results are in accord with the inferences of Havard & Wood (100) who considered the reduced urine flow to result from urinary salt loss and of Cutler *et al.* (101) who found that there was no decrease in urine volume when chlorothiazide was administered to diabetes insipidus patients if sufficient salt was administered to prevent negative balance. The proposal of Kennedy & Crawford (102) that the diuretics of this group produce their effects by a specific antagonism of the mineralocorticoids was presented largely without supporting data and is not easily reconciled with the findings of Earley & Orloff nor with those of Edmonds cited earlier (88).

The drugs as a group are rapidly absorbed from the gastrointestinal tract and rapidly excreted in the urine by a process involving active tubular secretion (74, 103). The latter process has been shown by stop-flow study to be localized in the proximal tubule (93) and to be inhibited by probenecid (74, 93) and, hence, presumably is that responsible for the secretion of

p-aminohippurate, diodrast, etc. Although earlier studies indicated that preventing the secretion of chlorothiazide did not reduce the diuretic effect when the drug was administered in large doses (74, 93), Beyer reports (104) that the action of the minimal effective dose is prevented by pretreatment with probenecid. This suggests that the concentration in the tubule lumen may be a critical factor in its activity.

The biochemical mechanism by which this group of compounds produces its effects is entirely unknown.

Mercurial Diuretics

The organic mercurials constitute the most effective diuretic agents known. Although the majority of those used clinically are substituted mercuripropyl derivatives with the following basic structure, R-CH_2-CHOY-CH_2-Hg-X (105, 106, 107), this configuration is not essential for diuretic activity; both mercuric chloride and mercuric cysteine (108) are diuretic as are certain organomercurials of dissimilar structure (109). The effect of variations in both the R and X groups which, in contrast to the OY group, may influence diuretic potency, solubility, and toxicity has been summarized in several articles (106, 107, 111). It is generally agreed that the terminal-C-Hg^+X^- group is largely responsible for diuresis, but whether this requires dissociation only to C-Hg^+ with one free mercury valence being made available (107) or rupture of the C-Hg bond releasing both valences (108) is the subject of much discussion (see below).

Mercury exerts its effect directly upon the renal tubular cells (112). Inhibition of sodium and chloride reabsorption follows accumulation of the derivative within the renal cortex (113, 114, 115). Chlormerodrin is secreted in the proximal nephron at a site co-extensive with that for PAH (116); it is probable that the other organic mercurials are also secreted by the same mechanism (117). Those which have been studied are excreted in the urine in large part as either the cysteine or acetylcysteine derivative of the parent compound (118, 119). The delay in diuresis which generally attends the administration of mercurials is not dependent upon a lag in either the uptake of mercury by the kidney or in its urinary appearance; considerable cortical accumulation (107, 114) as well as a high rate of excretion of the compound (115) may precede diuresis. Both Weiner *et al.* (120) and Campbell (117) consider that, in the chicken, mercury is transported into urine by a two-step process: tubule cell uptake which may be inhibited by probenecid and brom cresol green, and subsequent transfer into the urine.

Although probenecid interferes with both the excretion of mercury and its diuretic effect in the chicken (117), this has not been noted in other species. Kessler and associates (107) were unable to inhibit the secretion of *p*-chloromercuribenzoate, an inactive mercurial, with probenecid in the dog, and Weiner found no inhibition of the secretion of an active mercurial diuretic (119). A further demonstration of species differences is the obser-

vation that mercurials interfere with PAH transport in man, but not in the dog (121).

Inhibition of sodium and chloride reabsorption by mercurials is greater than that resulting from all other clinically useful diuretics (77). The increment in chloride excretion frequently exceeds that of sodium and is particularly notable in those edematous subjects in whom potassium loss in the urine is a prominent feature of the diuresis. This observation, as well as the occasional development of hypochloremic alkalosis following repeated injections of mercury, has led to the suggestion that the primary effect of the diuretic is on chloride transport, the diminution in sodium reabsorption being secondary to this phenomenon. Berliner (122) has pointed out the error in logic of this conclusion. The excess chloruresis observed is compatible with the view that proximal sodium reabsorption is primarily inhibited by mercurials, if one further assumes that the distal process whereby sodium is reabsorbed in exchange for potassium and hydrogen ions continues. Thus, if equivalent amounts of sodium and chloride are diverted from the proximal segment to the distal exchange site, one should expect chloride excretion to exceed that of sodium, the extra cation being made up by substitution of potassium, hydrogen, and ammonium ions. This would be particularly prominent in edematous subjects in whom the terminal exchange process is presumably stimulated by aldosterone. Predominant increases in potassium rather than sodium excretion are also observed with the chloruresis following administration of the thiazide derivatives in some edematous individuals and may be similarly interpreted (88). The observation of Giebisch (16) that chlormerodrin reduces the membrane potential of renal tubule cells in *Necturus* is consistent with, though not conclusive evidence for, the view that mercurials depress active sodium rather than chloride transport. Bisno *et al.* (123) have suggested that mercurials augment sodium excretion by increasing the passive flux of sodium from peritubular vessels into urine. Since the conclusion depends on interpretation of proximal tubule functions in stop-flow studies, it can not be accepted without reservation.

Mercurials inhibit the secretion of potassium in all species examined (31, 84, 124). In dog and man, if potassium excretion is initially elevated, mercurials uniformly lower the rate of excretion. On the other hand, if the initial rate of excretion is low, mercurial diuresis is associated with an increase in potassium excretion. The significance of this has been discussed in detail elsewhere (36, 84). Despite partial inhibition of the sodium-potassium exchange mechanism by mercury, the delivery of more sodium from the proximal segment to the exchange site provides the basis for increased secretion of potassium, albeit at less than the maximal rate.

Although it is generally agreed that changes in urine flow caused by mercurials are secondary to interference with solute reabsorption in the nephron, excluding a direct effect of the drug on water movement, consider-

able controversy exists as to the site of the diuretic effect. Depending upon the preconceptions of the investigator, the changes in urine flow induced by the mercurial during osmotic diuresis with and without antidiuretic hormone have been interpreted by some as deriving from the proximal nephron, by others from the distal nephron (94, 95, 125 to 133). Negative free water clearance has been reported to be unaltered by mercury in some studies, changed slightly in others. Similar results have been obtained with respect to the excretion of solute-free water.

Neither Miller & Riggs (131) nor Goldstein *et al.* (133) in carefully conducted studies observed any appreciable alteration in free water clearance during the superimposition of mercurials on maximal water diuresis in dog and man. The absence of a marked effect of mercurials on free water clearance contrasts with the results of studies with acetazoleamide and chlorothiazide. The administration of acetazoleamide is uniformly associated with an increase in free water clearance (134, 135), that of chlorothiazide with a fall (86, 94), indicating that they act on sodium transport at different sites in the nephron. The rise in free water clearance with acetazoleamide is indistinguishable from that observed during any nonspecific solute diuresis (136) indicating that there is no appreciable reduction of net solute reabsorption in those segments responsible for dilution of the urine. The increase in free water excretion is largely a consequence of a reduction in the back-diffusion of water attributable to the osmotic restraint of residual solute in the distal nephron (136, 137). The basis for the conclusion that chlorothiazide inhibits sodium chloride reabsorption in the diluting segment is discussed above. The mercurial effects are more difficult to interpret. Though consistent with a reduction in both proximal and distal reabsorption, the latter just sufficient to erase the increment in free water clearance generally provided by isosmotic solute diuresis, such a precise balance of the two effects is difficult to accept (133).

Diuresis caused by organic mercurials is strikingly potentiated by acidifying salts (138) and inhibited markedly by alkalinizing agents (139). Ethridge and his co-workers (139) demonstrated that the acidifying salts, ammonium chloride, ammonium nitrate, calcium chloride, and calcium nitrate, as well as phosphoric acid, when given in conjunction with salyrgan, effected a marked increase in the diuresis as compared with that due to the mercurial alone. In contrast the alkalinizing salts, potassium acetate, potassium bicarbonate, and sodium bicarbonate, all interfered with the diuretic response. The response was unmodified by the neutral salts, potassium chloride and sodium chloride.

Prior administration of sodium bicarbonate, though interfering with the natriuretic effect of mercurials, does not prevent the inhibition of potassium secretion (140). The potassium secretory system appears to be considerably more sensitive to mercury than is the system responsible for sodium chloride reabsorption since in normal dogs doses of mercury insufficient to increase sodium excretion diminish potassium secretion (141).

The increase in plasma chloride concentration, rather than changes in pH, has been thought to be responsible for the potentiating effect of acidifying agents (142). In support of this argument, Axelrod & Pitts (142) observed that respiratory acidosis, in contrast to metabolic acidosis, did not result in an augmentation of mercurial diuresis. Furthermore, the converse, hypochloremia, in edematous patients, is often associated with unresponsiveness to organic mercury. Although the filtered load of chloride may under certain circumstances condition the renal response to mercury, the "chloride load" hypothesis is inadequate to explain a number of observations. These have been discussed in detail by Levy *et al.* and Mudge & Weiner (108, 109). Potentiation occurs during chronic administration of ammonium chloride in the absence of significant hyperchloremia (143); ammonium nitrate augments the diuretic response despite absence of hyperchloremia (139); unresponsiveness to mercury is not observed when hypochloremia develops in hypokalemic alkalosis (144) but does occur in both metabolic (139) and respiratory alkalosis (145). Acetazoleamide which, in acute studies, does not appreciably affect the plasma chloride concentration has been reported to reduce the effectiveness of mercurials in both man (146) and dog (147). Finally, Levy *et al.* (108) have shown that sodium bicarbonate prevents mercurial-induced diuresis in dogs, even when the filtered load of chloride is artificially maintained at normal levels.

Most of the arguments relating to the site of action of mercurial diuretics have been based on histochemical data, alterations in water and electrolyte excretion, and effects on specific transport processes other than those for sodium and chloride in intact animals. Much of the earlier work has been reviewed in detail (109, 149) and will be commented on only briefly. It should be noted that neither the localization of a drug by histochemical techniques within a specific site in the nephron nor its interference with systems known to be present in specific segments, provides evidence regarding the site of inhibition of electrolyte transport by the agent in question. A drug may accumulate in a cell, enter the urine, and affect a luminal membrane transport process anywhere within the nephron. It may, after appearance in the urine, be reabsorbed more distally and exert its effect at another site. The available data relative to succinic dehydrogenase inhibition (150, 151, 152), sulfhydryl group concentration (153), and the transport of substances other than sodium and chloride (121, 125, 154) are probably of significance only with respect to the particular systems examined and may have no bearing on the diuretic action of the drug. Furthermore, conclusions relative to the site of action of mercurials based on the fraction of glomerular filtrate excreted in the urine are unwarranted, since partial inhibition of sodium transport throughout the nephron conceivably could result in similar data. On the other hand, there is no question that mercury exerts at least a portion of its effect in the distal segment in view of its interference with potassium secretion.

Vander *et al.* (148) and Kessler *et al.* (116) have utilized the stop-flow

technique in an effort to localize the site of action of mercury on sodium and chloride transport. Both groups have concluded that the major changes in water and electrolyte transport are limited to the proximal segment. This is based on two observations: (*a*) the minimal distal sodium concentration following mercury does not differ from that observed during control observations with mannitol alone, indicating to the investigators that distal sodium reabsorption is unaffected by mercury, and (*b*) the sodium concentration in the so-called proximal samples is greater with mercury than without, and the creatinine urine/plasma ratio is diminished, evidence of interference with both water and electrolyte reabsorption. The conclusion that the effect is exerted in the proximal tubule is based on the failure to demonstrate impaired sodium reabsorption in the distal tubule. However, the technique also failed to demonstrate that chlorothiazide inhibits distal sodium reabsorption, an action that fairly clearly exists (see above). As Kruhøffer (155) has stated, the findings in the distal effluent indicate that the concentration gradient against which sodium can be reabsorbed in the distal segment is not reduced by diuretic doses of mercury under the circumstances of the study, but do not prove that the reabsorptive capacity expressed as a rate is uninfluenced. Nevertheless, the contrast between the effects of mercurials and of chlorothiazide on water diuresis indicates considerable difference in their locus of action, and it appears fairly certain that a major part of the mercurial inhibition of sodium chloride reabsorption occurs in the proximal tubule. It may well be that partial inhibition of sodium transport throughout most of the nephron is effected.

The known affinity of inorganic mercury for sulfhydryl groups makes it probable that mercaptide formation is the principal reaction involving dissociable organic mercurials within the body. All active mercurials possess a C-Hg-X group which can provide at least one free Hg valence for this process; compounds of the structure R-C-Hg-C-R are not diuretic (149). The reversal and prevention of mercurial diuresis by the dithiol, 2,3-dimercaptopropanol (BAL), (156) and the failure of monothiols to do likewise (157) has been interpreted as indicating that the postulated sulfhydryl containing enzymes have a greater affinity for mercury than do the monothiols and a lesser affinity than do the dithiols (109, 149). The presumption is that BAL complexes with the mercurial *in vivo,* rupturing the inactive mercurimercaptide responsible for the diuresis.

Attractive as the sulfhydryl inactivation thesis may be, there is no direct evidence in support of it. There is little evidence that sulfhydryl-containing enzymes play a role in electrolyte transport. The early work implicating succinic dehydrogenase as the responsible energy source has been largely discarded (109, 158). Histochemical data correlating localized depression in sulfhydryl concentration within the nephron with mercurial administration are pertinent only to the extent (*a*) that blocking of sulfhydryl groups is responsible for the diuresis and (*b*) that blocking of sulfhydryl groups is limited to those involved in the transport process. On the other hand, the

lack of diuretic properties in *p*-chloromercuribenzoate (107), a known sulfhydryl inhibitor *in vitro,* does not exclude the participation of mercaptide formation, although it does exclude it as the sole factor responsible for diuretic activity. The recent observation (159) that *p*-chloromercuribenzoate both prevents and arrests diuresis resulting from active mercurials is strong evidence for sulfhydryl participation in the effect. If association or interaction with a receptor site adjacent to the sulfhydryl group is also necessary for diuresis, as suggested by both Mudge and Kessler and their associates (107, 109), formation of a *p*-chloromercuribenzoate mercaptide may prevent the active mercurial from engaging in this essential process.

Kessler *et al.* (107) and Mudge & Weiner (109) have proposed two alternative hypotheses concerning the mode of action of mercurials and the requirements for diuretic activity. Both groups consider accumulation, secretion, and mercaptide formation essential, although Mudge & Weiner caution against acceptance of the last as proven. Kessler *et al.* were able to show that the degree of accumulation of organic mercurials within the kidney varied and did not correlate with diuretic potency. They suggested that a specific steric configuration which permits a critical "lock and key" relationship between an active site in the kidney and the organic mercurial is a necessary requirement for diuretic potency. On the basis of an analysis of a number of organic mercurials, they concluded that diuretic activity requires that the compound contain a chain of not less than three carbons, a mercury atom at the end of the chain, and a hydrophilic group not less than three carbons away from the mercury. In their view, interference with electrolyte transport is dependent upon two conditions, combination of the free mercury valence of the terminal C-Hg^+ group with a sulfhydryl group and interaction of the hydrophilic group with an adjacent receptor site in the kidney. The thesis, which was recognized by the authors as being somewhat tenuous to the extent that they suggested an alternative hypothesis, is no longer acceptable. Numerous exceptions to the proposed configuration exist among organic mercurials, and mercuric chloride and mercuric cysteine are potent diuretics in the dog (109). Furthermore, the theory does not provide an explanation for the known effects of alterations in acid-base balance on the diuretic response.

Levy, Weiner & Mudge (108), on the other hand, concluded that diuretic potency requires rupture of the carbon-mercury bond, release of divalent mercury, and ionic interaction with two adjacent receptor sites within the kidney. They have suggested that the latter may involve association with a sulfhydryl group and an adjacent carboxyl or amino group. Since rupture of the carbon-mercury bond is a pH-dependent reaction, accelerated in acid solution, the release of ionic mercury within the body would be expected to be pH dependent. If the action of mercurials results from release of mercuric ion, rupture of the carbon-mercury bond would be accelerated in an environment of low pH either within the pertinent cells or the urine. In support of their theory they have observed that the diuretic

potency of both mercuric chloride and mercuric cysteine is unaffected by metabolic acidosis and alkalosis. All of the active organic mercurials which they studied liberate mercuric ions in an acid medium, whereas inactive mercurials with one exception do not. The acid-labile inactive mercurial did not appear in the urine following administration, suggesting that it was not transported to the proper place within the kidney to effect diuresis. Although they have been unable to detect inorganic mercury in the urine following injection of most of the active organic compounds, methodological limitations may account for this discrepancy, particularly since only a small fraction of the total mercury which accumulates in the kidney may be associated with the diuretic effect (115). Moreover, although most of the mercurials are excreted largely as the cysteine derivative of the parent compound (118), some, as is mercuric chloride, are excreted as mercuric cysteine (119). If this last is confirmed, it would constitute clear evidence of *in vivo* rupture of the carbon-mercury bond.

The hypothesis is attractive in that it suggests a reasonable explanation for the effects of alterations in acid-base balance on the diuretic response to the organic mercurials. It would be assumed that the enhanced diuretic activity in acidosis is attributable to acceleration of the production of ionic mercury by rupture of the carbon-mercury bond in an environment of lowered pH, whereas alkalosis would inhibit this reaction. However, it is difficult to specify an environment which undergoes pH change consistent in each case with the direction and magnitude of the modification of the diuretic effect. The plasma may be excluded since Mudge & Hardin (144) have shown that mercurial diuresis is not impaired in the alkalosis of potassium depletion, whereas the response to mercurials is depressed, markedly in man (146) and to a lesser extent in the dog (164), by acetazoleamide which has no effect on plasma pH (except for the reduction which results from impaired urinary acidification). Furthermore, it is not enhanced by respiratory acidosis which does reduce plasma pH (142, 160). Finally, Hilton (143) found that the potentiation produced by ammonium chloride was just as great when, as a result of compensatory mechanisms, plasma pH and bicarbonate had returned to normal as it was when the latter variables were low following the initial administration of ammonium chloride.

The possibility that it is modification of the pH of renal tubule cells which is critical to the efficacy of the mercurial diuretics is also not without objections, aside from the difficulty in specifying the extent and direction of changes in the intracellular pH. The pH of renal tubule cells is almost certainly lowered in respiratory acidosis which does not potentiate the action of mercurials (142, 160). The pH is also, in all probability, lowered in the alkalosis of potassium depletion (161, 162) which does not affect mercurial diuresis (144). On the other hand, the pH of tubule cells is probably more increased by the administration of potassium salts (163), which has not been found to suppress the effect of mercurials, than by the administra-

tion of sodium bicarbonate which has a very marked inhibitory action.

The possibility that the urine is the environment in which rupture of the carbon-mercury bond occurs is in accord with many but not all of the observations. The urine pH is not generally strikingly modified by either hypokalemic alkalosis or respiratory acidosis, nor is the diuretic response to mercurials. The urine pH is certainly elevated in alkalosis resulting from bicarbonate administration, and mercurial effects are suppressed. However, acetazoleamide is just as effective as the administration of bicarbonate in raising urine pH but much less so in preventing the action of organic mercurials.

This subject might be clarified by a careful quantitative re-evaluation of the effect of various conditions on the response to mercurial diuretics.

Cardiotonic Steroids

The cardiac glycosides are potent diuretic agents in congestive heart failure. Although both the glycosides and a number of the aglycones specifically interfere with cation transport in isolated tissues (166 to 169) and can be shown to exert direct renal tubular effects when injected into the renal circulation of experimental animals, they are not diuretic in the true sense in that their clinical effectiveness is limited to congestive heart failure. It is unlikely that the renal tubular effect demonstrated by Farber *et al.* (170) is a significant component of the diuretic action in heart failure. Such a thesis would presuppose unique changes in renal transport processes in congestive heart failure as contrasted with those in other conditions associated with edema (and in normals) which permit specific responsiveness to the digitalis-like derivatives. It is probable that diuresis, when observed, is secondary to changes in renal hemodynamic and cardiovascular function (171 to 173).

Despite this, the effects of these derivatives on electrolyte transport are of considerable interest. The drugs are important tools for examining the characteristics of cation transport. In a large variety of tissues it has been demonstrated that both the glycosides and, when studied, their aglycones specifically interfere with the active transport of sodium or potassium, or both. This was first established in the red cell by Schatzmann and has since been confirmed (165, 174 to 176) and extended to other tissues including the kidney (169). The molecular configuration necessary for activity has been studied in detail by Kahn & Acheson (175). The uniform interference with cation transport observed in the various tissues has led to the conclusion that all of the cells in question may transport cations by similar mechanisms, presumably involving linked exchange of sodium and potassium (177). Inhibition of potassium influx into red cells by the cardiotonic steroids is markedly diminished by increasing the concentration of potassium in the bathing medium (176). The kinetics of this process have been analyzed by Glynn (176) who considers the inhibition to be on a competitive basis. Inhibition of anion transport has also been observed; the accumula-

tion of PAH by renal cortical slices is diminished by strophanthidin (169), as is thyroidal iodide uptake (178). The inhibition in both instances appears to be related to the associated depression of cation transport since it can be minimized or prevented by increasing the potassium concentration of the medium and, in the case of renal cortical slices, reproduced by reducing intracellular potassium concentration by other means (169). The inhibition of chloride transport in gastric mucosa, on the other hand, may represent specific effects of the drugs on the chloride-transport process (179).

A direct renal tubular effect of digoxin was conclusively demonstrated by Hyman *et al.* (180). They observed a unilateral increase in sodium and water excretion following injection into the renal artery of the dog. These results have since been confirmed (181, 182). The observation by Schatzmann *et al.* (183) that ouabain interferes with net water movement in the proximal segment of *Necturus* is also interpreted as indicative of inhibition of sodium transport. Recently a detailed analysis of the effects of the aglycone, strophanthidin, on cation transport in the chicken kidney has been reported by Orloff & Burg (48). Injection of this compound into the renal portal venous circulation of one kidney results in a predominantly unilateral increase in sodium and chloride excretion and a fall in the excretion of potassium and hydrogen ions. Strophanthidin apparently interferes with all moieties of sodium reabsorption: reabsorption with chloride and exchange for potassium and hydrogen ions. The authors suggest that the effects are attributable to inhibition of the primary transport process which, in analogy to that proposed for frog skin (47), effects an exchange of sodium for potassium at the basal surface of the renal tubule cells.

In consonance with the observation of Glynn that the inhibitory effect of glycosides on red blood cells is reversed by increasing the potassium concentration of the medium, the diuretic effect of strophanthidin in the chicken and of ouabain in the dog is depressed by the administration of potassium salts (48, 181).

Wilbrandt has proposed (184) that the effect of digitalis-like substances is exerted through competition with adrenal mineralocorticoids, the latter acting as sites for the chelation of alkali metal ions on the membrane. Although this is an attractive hypothesis, Glynn (176) was unable to reverse the inhibition produced in human red cells by digoxin by the addition of large excesses of aldosterone and desoxycorticosterone, contrary to earlier findings (185). The effects of strophanthidin on the chicken kidney were not modified by large amounts of mineralocorticoids (48), although antagonistic effects in the rat have been reported (186).

The first metabolic effect of digitalis-like substances to be demonstrated was that strophanthidin had an ATP-sparing action in glucose-starved red blood cells (187). This is now known to be attributable to inhibition of an ATPase similar to that first found by Skou to be present in crab nerve (188) and since characterized in red blood cells by Post *et al.* (189). This ATPase requires sodium and potassium for activity, and its activity is

highly correlated with electrolyte transport in red cell ghosts (190). It seems probable that the renal effects are produced by a similar mechanism, and the presence in kidney of an enzyme with similar properties has been reported (191).

Antagonists of Aldosterone

Since the isolation and identification of aldosterone as the major mineralocorticoid of the adrenal (192), a tremendous literature has accumulated dealing with its physiologic effects, its role in various clinical disorders, and the factors governing its secretion. Several books and reviews may be cited (193 to 196). Space will not permit a review of the work relating to the effects of adrenal steroids on salt and water transport in the kidney. It is, however, pertinent to consider briefly the actions of a group of steroids which, because of their ability to reverse the actions of mineralocorticoids on renal sodium transport, have been introduced as possible diuretics.

The activities of the spirolactones, a series of compounds structurally related to aldosterone, were first described in 1957 by Kagawa *et al.* (197). These compounds have been shown to have the property of blocking, in competitive fashion, the effects of desoxycorticosterone and other mineralocorticoids on the excretion of sodium and potassium (198). Although there had been earlier instances reported in which steroids had been found to reverse the action of mineralocorticoids, in most of these studies it is highly probable that the effect of the antagonist was on glomerular filtration rather than transport by the tubules. This is clearly not the case with the spirolactones which, in general, have been found to have no effect on sodium or potassium excretion in the adrenalectomized or normal animal, but to decrease potassium excretion and increase sodium excretion in the presence of exogenous or endogenous mineralocorticoid (89, 197 to 199). Moreover, the effect of one of the series has been demonstrated in stop-flow studies in which glomerular filtration is excluded as a factor (200) and as an inhibitor of the effect of aldosterone on the sodium transport of isolated toad bladder (201). Thus, Vander *et al.* (200) were able, with SC 8109 [3-(3-oxo-17β-hydroxy-19-nor-4 androsten-17 α-yl) propionic acid γ-lactone], to reproduce in "normal" animals the modified stop-flow pattern which they found in adrenal insufficiency. It is noteworthy that this is the only instance in which such effects have been noted in animals neither given mineralocorticoids nor stimulated to secrete aldosterone by a low salt diet. However, the rather traumatic procedure involved in the surgical preparation for the stop-flow is sufficient to induce appreciable aldosterone secretion (202), and it is possible that some contraction of extracellular fluid may have been induced by the massive mannitol diuresis used for stop-flow studies.

In clinical use as diuretics, the spirolactones have proven considerably less effective than a number of other diuretic agents and their chief value seems to be in those relatively resistant patients in whom the administration of benzothiazide or mercurial diuretics produces a marked increase in

potassium excretion (89). In such individuals it has been assumed that the electrolyte excretion pattern is a manifestation of markedly increased aldosterone effects on sodium-potassium exchange, and this hypothesis appears to be confirmed by the fact that the excretion of potassium may be suppressed and that of sodium enhanced by the simultaneous administration of spirolactones.

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ENDOCRINE PHARMACOLOGY: SELECTED TOPICS[1]

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Pharmacology, as the science of the interactions of chemicals with living systems, embraces the study of hormones, naturally occurring chemicals vital to life and health and often important drugs as well. From the time of John Jacob Abel, pharmacologists have made notable contributions to endocrinology. Yet the study of hormones, aside from the catechol amines, is not the major field in present day academic pharmacology that it should be. Research on hormones is so important, so attractive, and so broad in its scope that it is not surprising that almost every medical discipline has shared in it. Other basic sciences that precede pharmacology in the medical curriculum ordinarily relieve departments of pharmacology of much of the responsibility for teaching endocrinology, and the subject of hormones as diagnostic and therapeutic agents is often left to the clinical departments. These are some of the factors that have helped to determine the present restricted position of endocrinology in pharmacology.

Despite the importance of the hormones in the spectrum of pharmacological science, it is realistic to recognize that progress in endocrinology is being continuously reviewed in the *Annual Reviews of Biochemistry, Physiology,* and *Medicine.* In the future it may be in order to reallocate endocrine topics among the four *Annual Reviews;* for the present a minimum of overlap has been the objective. Furthermore, limitations of space and of the capacity of the reviewer have prohibited a comprehensive survey. A number of areas of significant research have been omitted entirely, for example, the mechanism of action of insulin. Since selection was necessary, the topics chosen reflect, naturally, the interests of the reviewer. Three subjects, androgens, parathyroid hormone, and the hypothalamic secretory factor for ACTH, have been reviewed in considerable detail, although not exhaustively. Seven other topics have been dealt with briefly by citation of recent reviews and a narrow selection of current papers. The survey of the literature was concluded, for the most part, in August, 1960.

Androgens

The defining characteristic of an androgen, typified by testosterone, is

[1] Abbreviations used in this chapter include: CRF (corticotropin-releasing factor); DHA (dehydroepiandrosterone); HSF-ACTH (hypothalamic secretory factor for ACTH); MSH (melanocyte-stimulating hormone); TPN (triphosphopyridine nucleotide).

its ability to stimulate growth of the male secondary sex organs and development of masculine secondary sex characteristics. Testosterone and a number of other androgens also inhibit secretion of the hypophyseal gonadotropins, both follicle-stimulating hormone and interstitial cell-stimulating hormone, and hasten closure of the epiphyses. They stimulate general body growth, particularly the muscles and long bones, hence the term, anabolic steroid. In large doses, androgens may be palliative drugs in the treatment of inoperable breast cancer. Psychic and behavioral attributes associated with masculinity have also been ascribed to the androgens. *Androgens,* by Dorfman & Shipley (1), a comprehensive survey of the literature through 1953, provides a good basis for the appreciation of more recent developments.

No nonsteroidal androgen, with the possible exception of one phenanthrene derivative (2), has been discovered. Within the category of steroids, however, the structural requirements for androgenic activity are rather loose, with a broad range of specific activities. Therapeutic and commercial interest has led to a search for steroids that are anabolic or effective against breast cancer yet less androgenic than testosterone. Little effort has been devoted to the search for intrinsically more potent androgenic steroids, although there has been interest in the preparation of esters with a more extended duration of action. Testosterone and many other androgens are poorly active by the oral route of administration. On the other hand, methyltestosterone, highly active by mouth, is somewhat toxic to the human liver. The chemical-pharmacological approach toward the elimination of this undesirable side-effect will no doubt be pursued further, although the failure of the dog to exhibit the alterations of liver function tests seen in man may hamper laboratory investigations (3).

Biological assay.—Studies of the relation of structure and activity rest on methods of biological assay. The two principal classic types of assay method for androgenic activity are based on the growth of the comb of the capon, or more conveniently, the baby chick, and the growth of the prostate and seminal vesicles of the castrated rat. Munson & Sheps (4) have refined the baby-chick comb method by applying the test steroid directly to the comb in a minute volume (.01 ml.) of ethanol. The effect of as little as 0.05 μg. of the reference standard, androsterone, daily for seven days, was readily detectable. The delicacy of the method makes possible the assay of extremely weak androgens and of rare steroids available in very small quantity. The mean index of precision (λ) was 0.32.

Methods in current use for the evaluation of the anabolic activity of steroids use castrated male rats and are based on the gain in total body weight, the nitrogen balance, or the weight of the levator ani muscle. Because of its convenience, the levator ani method has been used most extensively. As usually conducted (5), after seven daily injections of test steroid, the ventral prostate and seminal vesicles as well as the levator ani muscle are weighed, giving indications of both androgenic and anabolic activities in the same test animal. Ratios of the two activities relative to a standard an-

drogen may be computed for the test steroid. In practice, statistical evaluation of the results often has been uncritical. Both specific and general criticisms have been levelled at the method. In contrast to the immediate cessation of growth of the prostate and seminal vesicles, the levator ani muscle continues to grow for several weeks after castration. The results may be misinterpreted, according to Gordan (6), if the assay is started immediately (5) rather than three weeks after castration as in the original method of Eisenberg & Gordan (7). Gordan (6) also recommended a longer period of administration in order to evaluate steroids with a delayed onset of action or cumulative effects. Kochakian & Tillotson (8) have observed that the effect of androgens on muscular growth varies not only from species to species but also from muscle to muscle within a species. The possibility must be considered that certain muscles, the levator ani among them, are sex-linked, and that growth of such muscles may be unrelated to more general anabolism. In man no thoroughly studied (nonphenolic) anabolic steroid has failed to be androgenic in a large proportion of patients if administered for a considerable period of time.

The procedure of Kochakian *et al.* (9) for demonstrating the anabolic activity of testosterone by production of a positive nitrogen balance in castrated male rats has been adapted for use as a quantitative biological assay method by Arnold *et al.* (10). A similar but more elaborate method in monkeys has been developed by Stucki *et al.* (11). Schedl *et al.* (12) and Liddle & Burke (13) have studied the relative anabolic activity of steroids in man under carefully controlled conditions. The retention of urinary nitrogen was measured during four-day periods of steroid administration in comparison with control periods preceding and alternating between treatment periods. Clear-cut differences and similarities in nitrogen balance that permitted gross comparisons between steroids were observed by Schedl *et al.*, but two-fold differences in dosage could not be discriminated. Liddle & Burke expressed their results as the difference in urinary nitrogen between the control and treatment periods. Forty-two tests with methyltestosterone resulted in rather widely scattered data, but there was a significant log dose-response curve. The index of precision (λ) was estimated at 0.4, indicating, as the authors acknowledge, that precise estimates of relative potency would require large numbers of observations.

Newly discovered effects.—Effects on androgen-dependent target organs include inhibition of the spontaneous contractions of the seminal vesicle of the castrated rat [Grunt and co-workers (14, 15, 16)] and increased rate of incorporation of labelled glucose and glucurone into the comb of the capon [Balasz *et al.* (17)]. New anabolic effects were reported by Kochakian (18), who showed that the negative nitrogen balance and loss in body weight produced by thyroxine in castrated rats could be overcome by simultaneous administration of testosterone propionate, and by Bajusz *et al.* (19) who were able to prevent, in part, the muscle atrophy following nerve regeneration after crushing injury in rats by feeding methyltestosterone or ethyl nor-

testosterone. Effects on bone were demonstrated by Kowaleski & Emery (20), who found that ethyl nortestosterone increased the rate of uptake of ^{35}S by the callus of healing fractures of rat humeri, and by Urist & Deutsch (21), who reported that androgen administration protects laying hens against the usual spontaneous osteoporosis. The hyperthermic effect in man of etiocholanolone, a major urinary steroid previously thought to be biologically inert, was first reported in 1957 (22). Further investigation of the structural requirements of a steroid for this effect will be hampered by the fact that of all animal species tested (mouse, rat, guinea pig, dog, cat, rabbit, monkey, and man) only man responded (23). Androsterone, the major androgen of human urine, has now been found by Hellman *et al.* (24) to be highly effective in reducing the level of serum cholesterol in patients with hypothyroidism, in other patients with hypercholesterolemia, and in normal subjects. The authors suggest that the elevated serum cholesterol in hypothyroidism may be related to the low levels of androsterone associated with this disorder. Weisenfeld & Goldner (25) found that several but not all androgenic steroids tested prevented the hyperglycemic effect of glucagon in human subjects. The most effective steroids possessed an alkyl substituent in the 17α-position, a structural characteristic of steroids with liver toxicity. No consistent correlation with androgenic or anabolic activity was observed, except that estrogens, progestagens, and glucocorticoids were inactive. Hilz & Utermann (26) have reported that testosterone ($10^{-7}M$) stimulates the oxygen consumption and synthesis of polysaccharide sulfate in the isolated normal rat aorta.

Relation of chemical structure to androgenic activity.—A comprehensive survey of the relative androgenic potency of steroids by different assay methods would help to clarify the currently confused status of the subject. Extensive new data and an exhaustive review, restricted to the effect of esterification of androgens with a wide variety of organic acids on the duration of androgenic action in the rat, have been published by Junkmann & Witzell (27).

Rosemberg *et al.* (28, 29) compared the androgenic activity of 4-androstene-3,17-dione with its 11β-hydroxyanalogue and the 9-hydroxy and 9α-halo-11β-hydroxy analogues by local application to the baby chick comb. The parent compound is a highly active androgen, while the 11β-hydroxy derivative is only very weakly active and, furthermore, exhibits a rather flat dose-response line. The 9α-F, 9α-Br, and 9α-Cl derivatives of 11β-hydroxy-androstenedione, also the 9β-11β-oxido and the 9α,11β-dihydroxy compounds were not detectably androgenic at the relatively high doses tested. These observations are in harmony with the unpublished results of Munson and Sheps, who found, in addition, that the 11-oxo- and Δ9-11 derivatives of highly active androgens were intermediate in potency between the parent androgens and the 11β-hydroxy derivatives. These characteristics of the structure-activity relationship of growth-promoting steroids are, of course, in marked contrast to the enhancing effect of 11-oxygen and 9α-halo substitution on

activity in the catabolic glucocorticoid series. An exception to these generalizations is the reported high androgenic activity of fluoxymesterone (9α-fluoro-11β-hydroxy-17α-methyl-19-nortestosterone) and its 11-oxo analogue by oral administration (30).

Alibrandi *et al.* (31) tested a series of acyl esters of testosterone and of dihydrotestosterone by the procedure of Hershberger *et al.* (5) and found that the esters were considerably more effective in oil solution than in aqueous suspension, whether administered orally or subcutaneously. Gleason & Parker (32) have reported a new long-acting androgenic steroid, the benziloyl hydrazone of testosterone heptanoate.

Anabolic steroids.—Extensive bibliographies of laboratory and clinical data on anabolic steroids have been published (12, 33). Berczeller & Kupperman (33) have discussed the principal anabolic steroids generally available for clinical trial. In addition to the classic testosterone esters and methyltestosterone, their list comprised methyl-androstenediol (5-androstene-3β,17β-diol), norethandrolone (17α-ethyl-19-nortestosterone), and norethandrolone phenylpropionate. Fluoxymesterone, methandrostenolone (17α-methyl,17β-hydroxy-1,4-androstadien-3-one), vinyl and ethynyl nortestosterone, and androstanazole [17α-methyl-17β-hydroxy-androstano-(3,2-c) pyrazole] should be added to the list. Contrary to the prediction from the levator ani tests in rats, Schedl *et al.* (12) found vinyl and ethynyl testosterone to be anabolic in man. Androstanazole was indicated to be a potent anabolic agent in rats both by nitrogen retention and levator ani test (10, 34). Lerner *et al.* (35) found nortestosterone benzoate, by the method of Hershberger *et al.* (5), to be much less (18 to 42 times) androgenic than testosterone propionate, but almost equivalent in its effect on the levator ani muscle. However, the dose-response curve for nortestosterone benzoate on levator ani weight was very flat.

The nature and mechanism of the anabolic effect of the androgenic and related steroids is still quite imperfectly understood 25 years after discovery of the phenomenon. A valuable paper by Kochakian & Endahl (36) clarifies the relationship between dosage of testosterone propionate, castration, food intake, and gain or loss of body weight in rats. The additive effect of testosterone proprionate and growth hormone on body weight gain previously shown in mice (37) was confirmed in castrated rats (38), indicating that the two anabolic actions are independent in both species. Kochakian & Costa (39) studied the effect of testosterone propionate in depancreatized castrated dogs given sufficient insulin to maintain life but not enough to prevent glycosuria. Administration of testosterone propionate, 25 to 100 mg./day, resulted in a decrease in excretion of glucose accompanied by retention of nitrogen and a continuous increase in body weight. The androgen is known to be without effect on the blood glucose level in dogs with pancreas intact. A review by Kochakian (40) on mechanisms of androgen action and two new papers (41, 42) are concerned principally with changes in enzyme activity of various tissues of animals treated with androgens. It seems fair to

say that the work on enzyme content of tissues stimulated by androgens has not yet developed any clear lead to the understanding of the mechanism of action of androgens.

Recently, a mixture of steroid esters formulated by Gassner *et al.* (43) for administration to feeding stock (steers) has been shown to act as an anabolic agent of potentially great economic importance. The mixture consists of testosterone enanthate 60 mg., 17α-hydroxy progesterone 60 mg., and estradiol valerate 24 mg., and is injected as a paste in polyglycol suspension into the ear of the animal. Estrogen could not be found in extracts of the edible parts of the carcass, and the delicacy of the bioassays employed excluded the presence of more than one-billionth part of the estrogen injected. The rather slow rate of disappearance of the androgen and the progesterone derivative from the circulation and the lack of delicacy of available tests for these steroids in tissues forced the conclusion that additional studies would be needed before a definitive decision could be made concerning the public health aspects of treatment of cattle with the anabolic steroid mixture (44).

Inhibition of gonadotropin secretion.—The ability to inhibit the secretion of anterior pituitary gonadotropins may be at least in part dissociated from other activities of androgens. Thus both fluoxymesterone (45) and 2α-methyldihydrotestosterone propionate (46) were shown to be as effective or more effective than testosterone propionate as anticancer agents, yet induced little or no reduction in gonadotropin excretion. On the contrary, although nortestosterone propionate resulted in a marked decrease in gonadotropin excretion (significantly less marked, however, than similar doses of testosterone propionate), it was virtually inactive as an anticancer agent (47). Epitestosterone propionate was inactive in both respects (48). An exhaustive compilation of tests, in animals as well as in man, for inhibition of gonadotropin secretion by various steroids would be helpful.

Nortestosterone propionate (*vide supra*) and methyl nortestosterone have recently been shown to inhibit gonadotropin secretion in man, as shown by reduction in urinary gonadotropins (47, 49, 50, 51). Both ethyl nortestosterone and ethynyl nortestosterone, the latter more than the former, inhibited gonadotropin secretion in young male rats as shown by the effect on spermatogenesis and on the weight and histology of the testis (52).

Glenn *et al.* (53) have shown that 9,11-epoxy-methyltestosterone, 11α-hydroxy-methyltestosterone, 2-methylnortestosterone, and 9α-F,11β-hydroxy-17-methyltestosterone are effective inhibitors of the growth of the Huggins tumor (a benign mammary fibroadenoma) in female mice, with little stimulating effect on the preputial glands (an indicator of androgenic activity in the female). All members of the group were relatively weak inhibitors of gonadotropin secretion as tested by the effect on growth of the testes.

Adrenal androgens.—The significance and even the identity of the adrenal androgens are not yet known with certainty. The term itself, adrenal androgens, has been used rather loosely to include steroids isolated from

adrenal extracts, steroids synthesized by adrenal tisue *in vitro,* steroids presumably secreted because they have been identified in adrenal venous blood, and androgenic metabolites (originating from adrenal precursors) found in peripheral blood, nonendocrine tissues, and urine or other excreta. In addition, species differences undoubtedly exist. All the so-called androgens of urine are present in urine as biologically inactive conjugates, sulfates and glucosiduronates; androgenic activity is manifested only after hydrolysis to the free steroid. Finally, a distinction should be made between adrenal androgens from normal and pathological adrenal glands. Short (54) has summarized the limited information through 1958 about the identity of the adrenal androgens.

A marked increase in the quantity of urinary dehydroepiandrosterone (DHA), a steroid known to be of exclusively adrenal origin, is commonly associated with but is not certainly responsible for the virilizing syndromes arising from adrenal pathology in women and children. DHA is approximately one-half as potent an androgen as testosterone when tested by direct application to the comb of the baby chick, but it is relatively weak in all other animal assays, and not clearly proved androgenic in man. The concentration of free, as distinct from conjugated, DHA in peripheral blood has not been determined either in adrenal virilism or in normal subjects.

In normal physiology the significance, if any, of the adrenal androgens is quite unclear. It is obvious that the adrenal androgens are inadequate to maintain the normal status of the secondary sex glands and secondary sex characteristics in castrated men and other male animals (55) or to exert well-defined effects in the normal female. However, there are recurring suggestions that an increased supply of adrenal androgen may be responsible for at least some cases of idiopathic hirsutism (56) and that adrenal androgens may be of importance in the maintenance of normal libido (57, 58). The normal rate of secretion of DHA has recently been estimated by Vande Wiele & Lieberman (59) to be to 25 mg. a day, an amount larger than for any other steroid secreted. Partly attributable to this high rate of secretion and partly to a rather slow rate of disappearance of its principal conjugate, the sulfate, from the circulation (half-life of 128 and 98 minutes respectively in two subjects) associated with a slow rate of clearance by the kidney (60), DHA is quantitatively the major conjugated 17-ketosteroid in peripheral plasma (61). DHA is also the third most prominent 17-ketosteroid of human urine. Furthermore, the two more abundant urinary 17-ketosteroids, androsterone and etiocholanolone, have DHA as their sole adrenal precursor. All these facts encourage further investigation of the adrenal androgens in general and DHA in particular.

The special pharmacology of DHA is poorly known, lending added interest to the recent investigation by Howard (62), in which the effects of subcutaneous compressed pellets of DHA, 11β-hydroxy-androstenedione, and corticosterone on the mouse adrenal gland and reproductive tract were compared. The reduction of adrenal weight by DHA resulted from an effect

on the X-zone exclusively, whereas the effect of corticosterone was on the zona fasciculata. DHA stimulated marked growth of the preputial glands and phallus of both sexes with only minimal stimulation of growth of the seminal vesicles. The effects of DHA were approximately duplicated by 11β-hydroxy-androstenedione, at a dose level three to four times as large as that of DHA. This work suggests that the various androgenic steroids may have differential effects on the different secondary sex organs.

Additional androgenic steroids have not been isolated from adrenal extracts since Short's review (54), which listed 4-androstene-3, 17-dione (androstenedione), adrenosterone, 11β-hydroxy-androstenedione, and 11β-hydroxy-epiandrosterone from cattle and hog adrenal extracts, and testosterone, DHA, and androsterone (incompletely identified) from human adrenal tumors. The androgenic activity of 17α-hydroxy-progesterone, also listed (54), is dubious.

Dorfman (63) has tabulated the androgens isolated from adrenal perfusates and incubates of slices and homogenates. Androstenedione, adrenosterone, 11β-hydroxy-androstenedione, and DHA make up the list. The same four steroids have been identified with varying degrees of certainty in adrenal venous blood of several species (54). In man the isolations have been from patients undergoing adrenalectomy for cancer or adrenal disorders. In addition, androsterone was identified in the adrenal venous blood of a woman suffering from virilizing adrenal hyperplasia (64). Short (54) identified androstenedione, 11β-hydroxy-androstenedione, and DHA in adrenal venous blood samples after administration of ACTH to three postmenopausal women with breast cancer. No testosterone, androsterone, or adrenosterone could be detected. Lombardo *et al.* (65) isolated 11β-hydroxy-androstenedione from adrenal venous blood of 10 of 12 elderly women with breast cancer, and DHA from only one of the 12. It has been suggested (59) that the age and debility of the patients were responsible for the failure to find DHA more consistently. Oertel & Eik-Nes (66) were unable to detect DHA with certainty in the adrenal venous blood of dogs, but, following perfusion of pregnenolone, high levels of DHA were found in the systematic plasma. They reported, furthermore, that the isolated hind leg of the dog converted pregnenolone and 17α-hydroxy-pregnenolone to DHA, obtained as DHA phosphate, which is also found in systemic plasma of normal human subjects infused with ACTH (67). It was concluded that DHA is formed outside the adrenal gland from pregnenolone and 17α-hydroxy-pregnenolone of adrenal cortical origin. This new concept will require further study and confirmation before it becomes generally acceptable.

Little is known about the normal levels of the free circulating androgens. Oertel & Eik-Nes found 1.4 μg. testosterone/100 ml. men's plasma after administration of chorionic gonadotropin, but none in normal plasma (68). Injected testosterone disappears from the circulation with great rapidity. Extracts of acidified human plasma contain DHA as the principal 17-ketosteroid, but this undoubtedly represents hydrolyzed DHA sulfate. An in-

genious new method by Finkelstein *et al.* (69) for the analytical determination of as little as 0.05 μg. of testosterone will permit a new attack on the problem. Plasma is extracted under conditions that protect steroid conjugates from hydrolysis, and the testosterone is isolated by paper chromatography and converted to estradiol and estrone by a placental enzyme preparation. The estrogens are purified by paper chromatography and estimated fluorometrically. In three normal men the values were 0.4, 0.2, and <0.1 μg./100 ml. In two normal women they were 0.1, and <0.1 μg./100 ml. In two women with virilizing syndromes, one with an adrenal adenoma and one with an ovarian hilus-cell tumor, values of 1.3 and 2.0 μg./100 ml., respectively, were obtained, suggesting that the steroid responsible for the virilization was testosterone. However, the data are too few to be certain that testosterone is consistently present in plasma from normal women.

On the basis of a variety of indirect evidence it has long been assumed that in the normal human male one-third of the urinary 17-ketosteroids represent testicular precursors and two-thirds represent adrenal cortical precursors. Relegating DHA and the 11-oxygenated 17-ketosteroids to the adrenal cortex exclusively, a reasonable estimate of the source of the principal urinary 17-ketosteroids, androsterone and etiocholanolone, has been one-half from the testis and one-half from the adrenal cortex. A bombshell has been dropped on this widely accepted concept by Vande Wiele & Lieberman (59), who concluded, with impressive although limited supporting data, that DHA from the adrenal cortex is the precursor of essentially all of the androsterone and etiocholanolone of human urine, men's as well as women's. This conclusion is based on the observation that when DHA-7-^{3}H was administered to two normal young men the specific activities of the three steroids (DHA, androsterone, and etiocholanolone) isolated from urine were essentially identical, indicating that dilution by unlabelled endogenous precursor was the same for all three steroids. The corollary conclusion, that testosterone contributes insignificantly to the urinary androsterone and etiocholanolone, is in apparent contradiction to the results of Harrison, Munson, and co-workers (70, 71, 72), who found that the excretion of urinary androgens (measured by biological assay) by elderly men with prostatic cancer was markedly reduced by orchiectomy. Additional unpublished work has extended the data to include 16 patients, all but two of whom exhibited a fall in androgen excretion after removal of the testes. The mean fall, including the two negative results, was 47 per cent, in excellent agreement with the assumption that half of the total urinary androgen in these men was of testicular origin. (The androgen content of suitably prepared urine extracts is essentially equivalent to the androsterone content.) A possible explanation of the disagreement in the results from the two laboratories is related to the decline in excretion of androgens and 17-ketosteroids with age.

Although it should be obvious that the fall in androgenic steroid excretion with age is so great in men that a decrease in secretion of the adrenal precursors must occur also, this inference has been emphasized only rarely

(73) in the literature. The less extensively documented fall in androgen excretion in elderly women and the hypothesis of Vande Wiele & Lieberman also require that urinary adrenal androgens must fall with increasing age. If the mean urinary androsterone in normal young men is taken as 6.0 mg./24 hrs. and the variability of the data of Lieberman & Vande Wiele allow for a 10 per cent testicular contribution to the urinary androsterone, then as much as 0.6 mg. of androsterone per 24 hours may be of testicular origin. In the elderly patients with prostatic cancer studied by Harrison & Munson, the mean daily excretion of androsterone before castration was only 0.96 mg., declining to 0.51 mg. after castration. The decrease resulting from castration (0.45 mg.) was therefore within reasonable agreement with the postulated testicular component of urinary androsterone of young men. This attempt to harmonize the two sets of data implies either that the decline in secretion of DHA with age is much greater than that for testicular testosterone, or that the relative size of the testicular contribution to the urinary androgens is much greater in men with prostatic cancer than in healthy men of the same age.

In patients with virilizing congenital adrenal hyperplasia attributed to a deficiency in the 21-hydroxylating enzyme, the secretion of 21-deoxycortisol and the excretion of 11-oxygenated-17-ketosteroids are greatly elevated, because of increased ACTH stimulation resulting from cortisol deficiency. Since, however, administration of 21-deoxycortisol does not lead to increased excretion of the 11-oxygenated-17-ketosteroids, the origin of the latter must be through a different pathway (74).

It now appears that in normal subjects, as well as in patients with congenital adrenal hyperplasia, androstenedione, rather than cortisol, 21-deoxycortisol, or 17α-hydroxy-progestrone, is the principal precursor of the urinary 11-oxygenated 17-ketosteroids (75, 76), which also suggests that the highly androgenic androstenedione may be a major adrenal secretory product. The studies of Wilson *et al.* (77) on the urinary steroids of four hypophysectomized women during ACTH administration also favor separate biosynthetic pathways for cortisol and adrenal precursors of 17-ketosteroids.

Wade *et al.* (78) found that rectal administration of cortisol was followed by a 100-fold increase in the excretion of 11β-hydroxy analogues of androsterone and etiocholanolone. The increase was abolished by administration of neomycin, implicating microbial action as the cause of the increased urinary metabolites, and suggesting the possibility that the minor normal conversion of endogenous cortisol to 11-oxygenated urinary 17-ketosteroids may occur in the intestine.

Antiandrogens.—Dorfman & Dorfman reviewed the literature on antiandrogens and described tests for such substances (79, 80). The comb of the baby chick was stimulated to grow by feeding testosterone with the diet or by a single subcutaneous injection of testosterone enanthate, and the test substance was given daily either by subcutaneous injection or by direct application to the comb. A number of estrogens, corticoids, and progesta-

tional substances, with 17α-ethynyl-nortestosterone the most active, appeared to inhibit the androgen-induced growth of the comb. In most cases the effect of the inhibitor was not clearly related to the dose. Lerner *et al.* (81) demonstrated the antiandrogenic effect of A-norprogesterone in both the chick and the rat. In confirmation of Hertz & Tullner (82), the carcinogen, methylcholanthrene, also inhibited the effect of androgen on the comb, and benzpyrene acted similarly (79). Previously shown to be antiandrogenic in the rat (83), 2-acetyl-7-oxo-1,2,3,4,4a,4b,5,6,7,8,9,10,10a-dodecahydrophenanthrene was found to be an active inhibitor in chicks. Dihydroisosteviol is another nonsteroidal substance reported to have an antiandrogenic effect on the chick comb (84).

Androgens in target organs.—Greer (85) injected rats with testosterone-^{14}C and plotted the disappearance rates from blood, muscle, and prostate gland. In both intact and castrated rats the ratio of activity, prostate to muscle, was usually about 2.5, and the activity found in the prostate gland was consistently although only slightly higher than that in the blood. Pearlman & Pearlman (86) infused male rats intravenously with 2.3 to 3.4 mc. of 4-androstene-3,17-dione-7-^{3}H (specific activity, 3.8 mc./mg.) at a constant rate for two to three hours. The ratio of radioactivity between extracts of ventral prostate and systemic plasma was 1.55; that between muscle and plasma was 0.72. In addition to androstenedione, radioactive metabolites, testosterone, androsterone, etiocholanolone, and androstanedione, possibly of significance for understanding the mechanism of action of androgens, were identified and quantitatively estimated in the prostate and plasma. Ofner *et al.* (87) identified radioactive 17β-hydroxy-1-androsten-3-one as a product of the incubation of testosterone-4-^{14}C with minces of human prostatic tissue.

Parathyroid Hormone

Research on parathyroid hormone, relatively neglected for a number of years, has flourished since 1955, and important advances have already been made in the purification of the hormone, quantitative methods for its biological assay, and studies of its sites and modes of action. There are several relatively recent reviews of earlier work (88 to 92), and a symposium (held in Houston, February, 1960) on current research on the parathyroid glands has been published in book form (93). The essay by Aurbach (94) is also of interest.

Purification.—The status of purification of the hormone was reviewed briefly in April, 1960 (95). The starting extracts for the current purified preparations include the classic hot hydrochloric acid extract (96), 0.1 N hydrochloric acid at 4°C. (97), hot acetic acid (98), and phenol (99). The first report of a substantial purification of parathyroid hormone was made by Rasmussen & Westall (100). They fractionated the ultrafiltrate of a hot dilute hydrochloric acid extract on Dowex 50 resin and obtained "Parathormone A," a product containing 100 units/mg., about six times the specific activity of the *United States Pharmacopeia* preparation marketed by Eli

Lilly and Co. Starting with the ultrafiltrate of the hot acetic acid extract of Davies & Gordon (98), Rasmussen isolated a different and apparently homogeneous peptide ("Parathormone B": specific activity, 220 units/mg.) by zonal electrophoresis on polyvinyl chloride (101). Friedman & Munson fractionated the hot acid extract with ammonium sulfate (102) and purified it by gradient elution on carboxymethylcellulose to obtain a final product containing 200 units/mg. (103). Application of the same procedure to the cold hydrochloric acid extract increased the specific activity to 1300 units/mg. (95). Aurbach (104) revived the use of phenol for preparing the crude extract, which he then fractionated with trichloracetic acid and organic solvents and purified by countercurrent distribution. The specific activity of the final product was estimated to be 2800 units/mg. Rasmussen & Craig (105) used a similar procedure and obtained a product of equivalent potency, identified as "Parathormone C." It appeared to be homogeneous by countercurrent distribution, ultracentrifugation, and dialysis. Amino acid analyses of Rasmussen's A, B, and C peptides indicated the presence of 16 or 17 amino acids; cysteine was not found consistently and methionine was present in all three (106). The calculated minimum molecular weights of peptides A, B, and C were, respectively, 3800 to 5600, 6512, 9267. Peptide C, although it has the largest molecular weight, also has the highest specific activity. The amino acid analyses also indicate that the three peptides do not represent mere polymers. Partial degradation of peptide C with accompanying loss of activity is the explanation favored by Rasmussen (106). Rasmussen announced the identification of the N-terminal amino acids of his peptides B and C at the First International Congress of Endocrinology, Copenhagen, in July 1960. Publication of the details of this work is awaited with interest.

Study of the stability of the purified preparations is complicated by variable times of onset and durations of action in test animals following subcutaneous injection. Rasmussen discovered that his preparations were reversibly inactivated by treatment with hydrogen peroxide (107), somewhat similar to the observations of Dedman *et al.* (108) on ACTH. Like ACTH in the hands of Dedman *et al.*, the activity was restored or possibly increased above the pretreatment value by treatment with cysteine at elevated temperatures. Others, however, found that the activity of crude as well as purified extracts, not intentionally inactivated, was enhanced markedly by the mere addition of cysteine without heat (109). Rasmussen has reported that the reversible inactivation with hydrogen peroxide is associated with the formation of methionine sulfoxide (106).

Biological assay.—For standardization of the *United States Pharmacopeia* extract, the venerable dog method is still official (110). It depends on the rise in blood calcium in intact dogs 18 hours after subcutaneous injection of extract. Too cumbersome for following the purification of extracts, it has been supplanted in the research laboratory by the method of Munson *et al.* (109, 111, 112), which employs young male rats given a calcium-free diet for four days then injected subcutaneously with extract immediately after para-

thyroidectomy. Six hours after parathyroidectomy the serum calcium of untreated rats has fallen to about 6 mg. per cent. The degree of maintenance of the serum calcium above this level (at six hours) bears a linear relationship to the log of the dose. The mean index of precision (λ) is 0.25 (109) considerably better than that of the method of Davies *et al.* (113), in which rats on a stock diet were parathyroidectomized three to 21 days before the assay and the serum calcium was determined 21 hours after injection. The six-hour time interval is also preferable to 21 hours for assessment of the potency of the shorter-acting purified preparations.

The limitations and shortcomings of the assay method of Munson *et al.* have been discussed in considerable detail (109). The addition of various substances (107, 109, 114) to parathyroid extract (cysteine, for example) or the employment of certain vehicles such as gelatin or oil (109, 114, 115, 116) greatly enhances the activity of crude as well as purified extracts although to varying degrees. The prevailing custom, in particular, of adding the adjuvant to the unknown only and not to the standard may lead to misinterpretation of the results. Although Aurbach (116) and Rasmussen (114) have stated that the activity of the *United States Pharmacopeia* extract is not affected by addition of cysteine or gelatin, the extensive data of Munson (109) can leave little doubt that marked enhancement of activity will be observed under carefully controlled conditions with adequate numbers of animals.

Clark *et al.* (117) have developed an assay method for parathyroid hormone based on the withdrawal of ^{45}Ca from the skeleton of the intact rat given ^{45}Ca six weeks prior to the assay. The assay method of Kenny & Munson (118) is based on the phosphaturic effect in rats during the first six hours after parathyroidectomy and subcutaneous administration of extract. Other assay methods published prior to 1958 were reviewed by Greep & Kenny (91) and Munson (109).

The small amounts of parathyroid hormone required to maintain the serum calcium of the parathyroidectomized rat and dog at a normal level indicate that the concentration of hormone in blood and urine is low (112, 119). A more delicate assay method than is yet available, coupled with a simple efficient method for concentrating the hormone from blood and urine, will be needed for adequate quantitative assay of these fluids. Davies (120) reported the detection of parathyroid hormone in the urine of hyperparathyroid patients. The urine was subjected to preliminary fractionation, and the resulting product was injected into intact mice. The presence of hormone was inferred from the increased excretion of inorganic phosphate in the urine. The precision of the assay method was low so that even with large numbers of mice the limits of error were broad. Buckner & Nellor (121) have attempted to estimate parathyroid hormone in serum of hyperparathyroid rats, but the positive results reported were borderline and of dubious quantitative significance.

Mode of action.—It is now well established, contrary to the classic

hypothesis of Albright & Ellsworth (122), that the parathyroid hormone acts to maintain the level of plasma calcium by some means independent of the kidney and of the level of plasma inorganic phosphate, almost certainly by a direct effect on bone. The evidence for this newer concept has been reviewed recently (95). The hormone also has a phosphaturic effect on the kidney, but this effect and the effect on bone appear to be relatively independent of one another. Additional effects of the hormone for which new evidence has been presented include increased reabsorption of calcium by the kidney (123), other renal effects involving bicarbonate, sodium, potassium, and chloride (124), stimulation of absorption of calcium by the gut (125, 126), effects on the distribution of phosphate between fluid compartments (127, 128, 129), and changes in the composition of milk in rats (parathyroidectomy is followed by decreased concentration of water and increased concentration of calcium; the hormone restores the composition toward normal) (95, 130).

New studies of direct effects on bone *in vitro* have been reported by Gaillard (131). Other recent work on the mechanism of action of parathyroid hormone on bone, with particular reference to the significance of citrate, was well summarized by Kenny (132), also by Freeman (133).

The general significance of the effect of parathyroid hormone on the excretion of phosphate by the kidney, now that this effect can no longer be accepted as the mechanism underlying regulation of the blood calcium level, has not yet been clarified. The reality of a phosphaturic action of parathyroid hormone is no longer in any doubt. It occurs with purified as well as crude preparations (104, 134, 135, 136) and is independent of changes in the glomerular filtration rate, which may be increased by crude extracts. The effect of the hormone when injected intravenously is extremely rapid. Gershberg *et al.* (137) measured glomerular filtration rate, renal plasma flow, and serum and urinary inorganic phosphate before and at short intervals after intravenous injection of *United States Pharmacopeia* parathyroid extract in 42 normal human subjects and patients. In all cases except two with hyperparathyroidism, the percentage of filtered phosphate reabsorbed fell within 15 minutes after the injection. Beutner & Munson (138) studied the effect of parathyroidectomy and parathyroid extract on the urinary phosphate in unanesthetized rats infused with physiological salt solution in order to provide urine samples at frequent intervals. A significant fall in urinary phosphate occurred within 25 to 40 minutes after parathyroidectomy. One to two hours later, after the urinary phosphate had reached a very low level, parathyroid extract was injected intravenously. In every case a rise in urinary phosphate occurred within eight minutes. The rapidity of the response to the hormone in both rat and man suggested that there is a direct effect on the renal tubule. Lavender (136) and Pullman *et al.* (139) injected small doses of purified parathyroid hormone into one renal artery of the intact dog while collecting urine from the two kidneys separately. In the majority of dogs studied the phosphaturic effect was confined to the injected kidney, direct evidence for a local action of the hormone on the kidney.

The site of action of parathyroid hormone within the renal tubule is in

dispute. Pitts *et al.* (140) and Malvin *et al.* (141) localized the proximal tubule as the principal site of phosphate reabsorption by the mammalian kidney; active secretion of phosphate was not observed. Nicholson & Shepherd (142) studied the sites of phosphate transport in the tubule of the dog with the aid of differential nephrotoxic agents and concluded that there was active secretion of phosphate in the distal tubule as well as reabsorption in the first third of the proximal tubule. Studies with parathyroid extract (143) in similarly treated dogs seemed to show that the principal mode of action of the hormone on the kidney was to stimulate active secretion of phosphate in the distal tubule with little or no effect on reabsorption. On the other hand, Samiy *et al.* (135), in a preliminary report of experiments with the stop-flow technique in parathyroidectomized dogs, found no evidence of net active secretion anywhere along the tubule. The principal site of reabsorption of phosphate, about which there is no controversy, was again found to be in the proximal tubule, and it was concluded that the effect of administration of purified parathyroid hormone was exerted in both proximal and distal segments. Samiy *et al.* (unpublished) have concluded from stop-flow experiments on additional parathyroidectomized dogs that the major action of the hormone is on reabsorption in the proximal tubule. It may be difficult to resolve the differences in the results obtained by the two techniques. There is little question that active secretion of phosphate occurs in the kidney of the chicken (144), but the work of Nicholson & Shepherd is virtually alone in concluding that a similar phenomenon exists in mammals. Komarkova *et al.* (145) observed a marked increase in the citric acid content of the rat kidney two hours after subcutaneous injection of a small dose of crude parathyroid extract. Since there was little or no effect one hour after the injection, it might appear that the effect was too slow to be of importance in the more rapid phosphaturic effect just described. However, the route of administration may have been responsible for the delay in response, and for clarification it would be necessary to measure the time course of both effects simultaneously after intravenous as well as subcutaneous administration.

The possibility that there is more than one parathyroid hormone has been suggested repeatedly, particularly with respect to the separate effects on bone and on the kidney. The purified hormone preparations of Aurbach, Rasmussen, and Munson have all been found to possess both calcium-mobilizing and phosphaturic activities (104, 134, 135, 136). Although the eventual separation of two hormones is still conceivable and can hardly be eliminated as a possibility until the native peptide has been synthesized, the evidence available at present favors the possession of both activities by a single hormone. Purified preparations have not yet been tested for the other less thoroughly studied effects enumerated above.

Hypothalamic Secretory Factor for ACTH

The biosynthesis and secretion of two of the three major classes of adrenal cortical steroids, glucocorticoids and the adrenal androgens, are dependent on and regulated by the anterior pituitary through ACTH. The

third major type of adrenal cortical activity, regulation of electrolyte metabolism, to which both aldosterone and the native glucocorticoids contribute (146), is at least partially dependent on ACTH. The rate of secretion of ACTH itself is held in check by the level of circulating glucocorticoids, and thus the entire system is regulated in part by a feed-back mechanism familiar in endocrinology. However, a wide variety of changes in the external and internal environment can also affect the secretion of ACTH without being mediated through alterations in the blood cortical steroid level. Most, if not all of the known effective stimuli for increased ACTH secretion are also "stressful." Today, it is less certain than it seemed 10 years ago that the hypophyseal and adrenal cortical responses to stresses are beneficial or even physiologically significant, but, notwithstanding, the phenomenon is a striking one that has attracted widespread attention and critical analysis.

Much indirect evidence has accumulated in support of the concept that stressful stimuli act through the central nervous system, more specifically the hypothalamus, which secretes a neural hormone into the hypothalamico-hypophyseal portal vessels to reach the anterior pituitary where it increases the rate of secretion of ACTH. The classic evidence in support of this concept was marshalled by Harris (147). More recent developments have been thoroughly and critically summarized from five differing points of view by Sayers *et al.* (148), Saffran & Saffran (149), Ganong & Forsham (150), Fortier & de Groot (151), and Vogt (152). Additional useful reviews were cited by these authors.

The experiments of Egdahl (153) may necessitate modification of this almost universally accepted hypothesis. Leaving the pituitary intact, the brain of the dog anterior to the inferior colliculus, including the hypothalamus and median eminence, was removed. During the three to five days of subsequent survival the adrenal venous 17-hydroxy-corticosteroids were measured under resting conditions, following a severe burn applied to a leg, and after a large dose of ACTH. The resting level of 17-hydroxy-corticosteroid secretion was found to be much higher than that of intact dogs, and the absence of the hypothalamus did not prevent increased steroid secretion following the burn, although this increase was less than that produced in the same dogs by ACTH or that following a burn in anesthetized intact dogs. The stimulus from the burned leg was transmitted over neural pathways rather than through the circulation, since the response was abolished by nerve transection and was unaffected by occlusion of the veins from the leg (154). Egdahl (153) concluded, therefore, that afferent neural stimuli traveled from the leg to the midbrain, where they stimulated elaboration of a neural hormone transmitted to the anterior pituitary through the systemic circulation. Absence of a center in the forebrain that normally inhibits ACTH secretion was suggested to explain the high resting steroid secretion in the operated dogs. Further investigation of the postulated hypothalamic secretary factor for ACTH, based on extensive evidence from many laboratories, is not likely to be abandoned immediately as a result of Egdahl's ex-

periments. When they are confirmed and extended, however, it must be acknowledged that at least one type of stress can result in increased ACTH secretion in the absence of the hypothalamus.

Biological test systems.—Whether the postulated hormone is identified as one of the known constituents of the hypothalamus or whether it exists as an unknown component of hypothalamic extracts, a specific biological test is needed to establish this identity or to help in the isolation of the new substance. All of the tests that have yet been devised are open to criticism of one type or another. Since there are many drugs and many experimental procedures that provoke increased secretion of ACTH indirectly through the mediation of the hypothalamus, the fundamental requirement for specificity in a test system is that the hypothalamus must be removed or damaged or its function must be inhibited in a manner adequate to distinguish between nonspecific stimuli that act on the hypothalamus and the hormone, which acts directly on the anterior pituitary. There are four principal categories of tests in current use, each of which attempts to fulfill this requirement in a different way. In one, rats with extensive median eminence lesions are used. In another, hypothalamic function is inhibited by administration of cortisol or other glucocorticoid substance. In a third, suitable drugs are used as hypothalamic inhibitors. Finally, rat anterior pituitary separated from the hypothalamus is incubated *in vitro* with test substances. In all the tests a positive result requires the demonstration of increased secretion of ACTH, either inferred from adrenal cortical responses in the original test animal or by assay of the blood or incubation medium in a second test system. A second requirement of a specific test system is that it must be able to discriminate between ACTH and the hypothalamic secretory factor for ACTH. Before consideration of these test systems in detail, the justification for their use will be examined.

Studies of the location and the extent of lesions in the hypothalamus that are effective in eliminating the ACTH response to nonspecific stimuli have been reviewed previously (148, 150). In the rat the most effective lesion is a large, more or less obliterative lesion of the median eminence introduced by McCann (155). In spite of interference with the blood supply to the pituitary, this lesion does not prevent increased ACTH secretion in response to the injection of hypothalamic extracts (156, 157).

When a sufficiently high dose of a glucocorticoid is administered to rats the ACTH response to nonspecific stimuli may be markedly inhibited or abolished. Porter & Jones (158) demonstrated that doses of cortisol that are effective in inhibiting the response of rats to nonspecific stimuli permit ACTH secretion following injection of extracts of hypophyseal portal blood. Other evidence had suggested that both the hypothalamus and anterior pituitary are directly inhibited by glucocorticoids (150).

A number of drugs have been shown to inhibit the ACTH response to stressful stimuli under specified experimental conditions. The drugs, paradoxically, are capable of acting as nonspecific stimuli for ACTH secretion under

third major type of adrenal cortical activity, regulation of electrolyte metabolism, to which both aldosterone and the native glucocorticoids contribute (146), is at least partially dependent on ACTH. The rate of secretion of ACTH itself is held in check by the level of circulating glucocorticoids, and thus the entire system is regulated in part by a feed-back mechanism familiar in endocrinology. However, a wide variety of changes in the external and internal environment can also affect the secretion of ACTH without being mediated through alterations in the blood cortical steroid level. Most, if not all of the known effective stimuli for increased ACTH secretion are also "stressful." Today, it is less certain than it seemed 10 years ago that the hypophyseal and adrenal cortical responses to stresses are beneficial or even physiologically significant, but, notwithstanding, the phenomenon is a striking one that has attracted widespread attention and critical analysis.

Much indirect evidence has accumulated in support of the concept that stressful stimuli act through the central nervous system, more specifically the hypothalamus, which secretes a neural hormone into the hypothalamico-hypophyseal portal vessels to reach the anterior pituitary where it increases the rate of secretion of ACTH. The classic evidence in support of this concept was marshalled by Harris (147). More recent developments have been thoroughly and critically summarized from five differing points of view by Sayers *et al.* (148), Saffran & Saffran (149), Ganong & Forsham (150), Fortier & de Groot (151), and Vogt (152). Additional useful reviews were cited by these authors.

The experiments of Egdahl (153) may necessitate modification of this almost universally accepted hypothesis. Leaving the pituitary intact, the brain of the dog anterior to the inferior colliculus, including the hypothalamus and median eminence, was removed. During the three to five days of subsequent survival the adrenal venous 17-hydroxy-corticosteroids were measured under resting conditions, following a severe burn applied to a leg, and after a large dose of ACTH. The resting level of 17-hydroxy-corticosteroid secretion was found to be much higher than that of intact dogs, and the absence of the hypothalamus did not prevent increased steroid secretion following the burn, although this increase was less than that produced in the same dogs by ACTH or that following a burn in anesthetized intact dogs. The stimulus from the burned leg was transmitted over neural pathways rather than through the circulation, since the response was abolished by nerve transection and was unaffected by occlusion of the veins from the leg (154). Egdahl (153) concluded, therefore, that afferent neural stimuli traveled from the leg to the midbrain, where they stimulated elaboration of a neural hormone transmitted to the anterior pituitary through the systemic circulation. Absence of a center in the forebrain that normally inhibits ACTH secretion was suggested to explain the high resting steroid secretion in the operated dogs. Further investigation of the postulated hypothalamic secretary factor for ACTH, based on extensive evidence from many laboratories, is not likely to be abandoned immediately as a result of Egdahl's ex-

periments. When they are confirmed and extended, however, it must be acknowledged that at least one type of stress can result in increased ACTH secretion in the absence of the hypothalamus.

Biological test systems.—Whether the postulated hormone is identified as one of the known constituents of the hypothalamus or whether it exists as an unknown component of hypothalamic extracts, a specific biological test is needed to establish this identity or to help in the isolation of the new substance. All of the tests that have yet been devised are open to criticism of one type or another. Since there are many drugs and many experimental procedures that provoke increased secretion of ACTH indirectly through the mediation of the hypothalamus, the fundamental requirement for specificity in a test system is that the hypothalamus must be removed or damaged or its function must be inhibited in a manner adequate to distinguish between nonspecific stimuli that act on the hypothalamus and the hormone, which acts directly on the anterior pituitary. There are four principal categories of tests in current use, each of which attempts to fulfill this requirement in a different way. In one, rats with extensive median eminence lesions are used. In another, hypothalamic function is inhibited by administration of cortisol or other glucocorticoid substance. In a third, suitable drugs are used as hypothalamic inhibitors. Finally, rat anterior pituitary separated from the hypothalamus is incubated *in vitro* with test substances. In all the tests a positive result requires the demonstration of increased secretion of ACTH, either inferred from adrenal cortical responses in the original test animal or by assay of the blood or incubation medium in a second test system. A second requirement of a specific test system is that it must be able to discriminate between ACTH and the hypothalamic secretory factor for ACTH. Before consideration of these test systems in detail, the justification for their use will be examined.

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A number of drugs have been shown to inhibit the ACTH response to stressful stimuli under specified experimental conditions. The drugs, paradoxically, are capable of acting as nonspecific stimuli for ACTH secretion under

other experimental conditions. In several cases, morphine (159, 160) and reserpine (161) for example, the initial injection of the drug provokes increased ACTH secretion, but adaptation occurs after treatment for several days, and the stimulating action is replaced by inhibition. Kitay *et al.* (162) reported that repeated administration of epinephrine or reserpine eventually resulted in a decreased amount of ACTH in the pituitary and suggested that the smaller supply of ACTH was responsible for the failure to respond to normal stimuli. They did not demonstrate that a moderate reduction in pituitary ACTH content necessarily results in decreased sensitivity to stimulation of ACTH secretion. Furthermore, the assays for rat pituitary ACTH were based on the *in vitro* adrenal slice method (163, 164), which estimates approximately three to 10 times as much ACTH in rat pituitary tissue as is indicated both by the classic adrenal ascorbic acid method (165, 166, 167) and the new method based on plasma corticosterone (168).

Morphine is the drug in current use for inhibiting hypothalamic function in tests for hypothalamic hormone. It is administered to rats previously anesthetized with pentobarbital; used in this way, it does not itself stimulate ACTH secretion (159, 160) and inhibits the ACTH response to a wide variety of stressful stimuli (159, 169, 170). However, increasing the intensity of the stimulating procedure or the dose of the nonspecifically stressful substance will overcome the morphine inhibition (159, 160, 171). Nalorphine antagonizes the inhibition of ACTH secretion by morphine in anesthetized rats (172) as well as the stimulation of ACTH secretion in unanesthetized rats (173).

In the test system used by Royce & Sayers (156), young male rats that excrete 75 ml. or more of urine in the 24 hours following electrolytic destruction of the median eminence are selected for test 48 hours after the operation. The decrease in adrenal ascorbic acid 30 minutes after removal of one adrenal and intravenous injection of extract is taken as a measure of the ACTH secretion produced by the extract. Corrections for contamination of the extract are obtained by separate bioassays for vasopressin (174) (which, in high doses, stimulates ACTH secretion in the lesioned rat) and ACTH (175). In the hands of Royce & Sayers, there was no significant difference between the response to ACTH in rats 48 hours after median eminence lesions and that in 24-hour hypophysectomized rats, while others (157, 176) required three or more times the dose of ACTH in the lesioned rat to produce the same effect as that obtained in the hypophysectomized rat.

The procedure just described is a simplification of the rather cumbersome method recommended earlier by Sayers (177), in which extracts were tested in decerebrate rats with median eminence lesions following bilateral adrenalectomy two or more weeks earlier to allow time for development of the increased level of blood ACTH required for quantitative assay. After injection of the test substance the concentration of ACTH in the blood was estimated by bioassay in hypophysectomized rats.

In the procedure of Porter & Jones (158), intact rats are pretreated

with a large dose of cortisol acetate (6 mg./100 gm.) four hours prior to removal of one adrenal gland and intravenous injection of test extract. A second injection is made 30 minutes later, and 60 minutes later the second adrenal is removed. As in the procedure of Royce & Sayers (156), the effect on ACTH secretion is indicated by the difference in ascorbic acid concentration between the two adrenal glands.

The assay system developed by Leeman (178) and Leeman & Munson (179) involves the use of three procedures in each of which reduction of adrenal ascorbic acid of rats is used as the indicator of increased ACTH in the circulation. First, the intravenous dose-response curve for the extract in intact rats anesthetized with pentobarbital is obtained. Second, the relative effect of a submaximal dose of the extract in the anesthetized rats is determined with and without pretreatment with morphine. Lack of inhibition of the effect by morphine is an indication of the absence of nonspecific active substances, and the extent of noninhibited effect is taken as a measure of the amount of hypothalamic hormone. Use of submaximal doses of extract is important to avoid misinterpretation of a strong nonspecific stimulus that has surmounted the morphine inhibition for a hormonal effect that is not inhibited by morphine. The extent of contamination of extracts with ACTH must be measured in hypophysectomized rats in the usual way (175, 180, 181). The intact rat given pentobarbital and morphine cannot discriminate between hypothalamic hormone and ACTH; indeed, the response to intravenous ACTH was quantitatively identical in the three types of test rat, within the limits of experimental error. Extracts free of nonspecific stimulating substances and ACTH may be assayed reliably in anesthetized intact rats without morphine.

Guillemin *et al.* (176) have modified this test system in several important ways. Fifteen minutes after intravenous injection of extract into rats pretreated with pentobarbital and morphine, a blood sample is collected for fluorometric estimation (182, 183) of plasma corticosterone. Previous studies (168) had shown that a maximum effect of ACTH was attained in hypophysectomized rats 15 minutes after injection, with a linear log dose-response curve. A similar relation held between the log dose of a purified posterior pituitary extract (Fraction D) and the response (176). Determination of plasma corticosterone was preferred to adrenal ascorbic acid as a measure of ACTH secretion because the former is more obviously related to the principal function of the adrenal cortex than the latter, and because the minimum dose of extract required for an effect on plasma corticosterone appeared to be lower than that for an effect on adrenal ascorbic acid by a factor of 20 or more. (The ratio of the two minimum effective doses for ACTH was not consistently greater than two.) One criticism of the test system, its response to vasopressin (minimum effective dose: 100 milliunits), and presumably other nonspecific substances given in a large enough dose to surmount morphine inhibition, could be met by demonstrating equivalent responses to extract by test rats with and without

morphine, as in the method of Leeman & Munson. The methods of Royce & Sayers (156) and of Porter & Jones (158) are open to a similar criticism and a similar correction.

The *in vitro* assay system originating with Saffran and co-workers is based on the increased amount of ACTH found in the medium following incubation of rat pituitary tissue with test extracts. As described in its most recent form by Schally *et al.* (184), after "preincubation" of pairs of vessels, each containing half of a rat anterior pituitary, for one hour, the media are discarded, fresh medium alone is added to the control vessel and medium plus extract to the other. At the end of one additional hour of incubation, the relative ACTH content of the media in the paired control and experimental vessels is estimated by bioassay. A ratio > 1.0 at P.05 is considered to indicate the presence of hormone in the extract. (In order to correct for contamination of the medium with ACTH from the extract, extract is added to the control vessel at the end of the second incubation). Under favorable conditions a log dose-response relationship has been demonstrated (185), but apparently this has not been a consistent finding. Ordinarily, the minimum effective dose of the extract is estimated by testing serial dilutions. The "preincubation" part of the procedure was introduced in order to decrease and make more consistent the amount of ACTH released by control tissue. The ACTH in the media is assayed by an *in vitro* method (163), in which the production of ultraviolet-absorbing material by incubated quartered rat adrenals is linearly related to the log dose of ACTH. The *in vitro* system used in the laboratory of Guillemin (186) for assay of hypothalamic hormone is essentially identical with the one described by Schally *et al.* (184), except for omission of preincubation.

The assay system just presented has been subjected to several criticisms. The *in vitro* assay method for ACTH responds nonspecifically to plasma and plasma fractions (187) although their effects, unlike those of ACTH, are not related to the dose (162). Furthermore, the method estimates the ACTH content of rat pituitary tissue to be three to 10 times greater than that found by other methods (165, 166, 167), raising the possibility that part or all of the increase measured may not be ACTH. However, as exemplified by Swingle *et al.* (188, 189), increases in the ACTH content of the medium of incubated rat pituitary are not dependent on the *in vitro* method for ACTH assay; they may also be demonstrated by the classic adrenal ascorbic acid method. Lack of precision of the Saffran method seriously restricts its usefulness for quantitative bioassay. However, the most important limitation of the method is its lack of specificity. Fortier & Ward (190) found activity in the peripheral plasma of rats with no correlation between the effect and the endocrine status of the animal. Saffran (185) obtained positive results with synthetic oxytocin, Pitressin, purified lysine vasopressin, and synthetic arginine vasopressin, and, in addition, extracts of brain cortex, substance P from horse and cow gut, and hydrolysates of substance P and of Pitressin were found by others (186, 189)

to be active. Saffran (185) suggested that the scattered activities might be explained as an overlap of activities between peptides of similar structure, like the analagous behavior of oxytocin and vasopressin in other test systems. A more skeptical position was taken by Fortier & Ward (190), who stated that a positive result may mean either that the added material (*a*) contains ACTH, (*b*) potentiates the action of ACTH in the assay method for ACTH, (*c*) protects ACTH from inactivation, (*d*) actually stimulates ACTH release, or a combination of these effects. Items (*a*) and (*b*) were eliminated by adding the test material to the control medium at the end of the incubation before assaying for ACTH. Item (*c*), also suggested by Barrett & Sayers (191) was shown not to occur in experiments by Saffran (185) and by Guillemin & Schally (192), who demonstrated that no loss of ACTH activity occurred during the incubation period, either with or without the addition of extract.

The significance of the various purified extracts of hypothalamus or posterior pituitary must be evaluated in the light of the limitations of the test systems employed for identification and estimation of relative potency.

Purification of extracts.—Saffran *et al.* (193) were the first to obtain a substantial purification of the presumed hypothalamic secretory factor for ACTH. They applied the term corticotropin-releasing factor (CRF) to the postulated neural hormone. The term was appropriate for the *in vitro* test system employed; it has been used widely and is generally accepted. The reviewer prefers hypothalamic secretory factor for ACTH (HSF-ACTH) because, although somewhat more cumbersome, it denotes the physiological activity of the factor under study without restriction to a particular assay system.

Protopituitrin, an intermediate fraction in the commercial preparation on posterior lobe extracts, was chosen for further study by Saffran and coworkers because of its high CRF activity and because it was more readily available than hypothalamic extracts as starting material. Schally *et al.* (184) purified protopituitrin by serial paper chromatography in four different solvent systems. In this way an active fraction was repeatedly obtained which was separated from ACTH, oxytocin, and arginine and lysine vasopressins, and which was stated to be active in the *in vitro* test system at 0.001 μg., less than one-tenth the amount required of any other product yet reported. Amino acid analyses of two such products showed the presence of the amino acids of lysine vasopressin, plus serine, histidine, alanine, and arginine. Quantitatively, the two preparations differed considerably in per cent amino acid composition, suggesting, since they were equipotent, that neither one was homogeneous. The yield from 3 gm. of protopituitrin was 0.6 mg., an insufficient quantity for extensive further studies.

In more recent collaborative work by Schally & Guillemin (194, 195, 196) the paper chromatographic approach to purification has been superseded by procedures more adaptable to large-scale preparations. A continuing problem has been the separation of CRF from other peptides, partic-

ularly vasopressin and melanocyte-stimulating hormone (MSH), which when they themselves are tested in pure condition are not without effect on the *in vitro* test system.

Protopituitrin or "pitressin intermediate" was first treated three times with oxidized cellulose to remove the major portion of ACTH present. Inactive material in an acetic acid solution of the product was precipitated by acetone. Addition of ether precipitated the activity, which was applied to a column of carboxymethylcellulose. Eluates were tested for CRF by the *in vitro* method and also for MSH, ACTH, oxytocin, and vasopressin. Oxytocin, β-MSH, arginine vasopressin, and ACTH were well separated, but the CRF activity resided in a single peak that also contained α-MSH and lysine vasopressin. The eluates containing CRF were subjected to countercurrent distribution and, in confirmation of Gros & Privat de Garilhe (197), two peaks were separated, both containing CRF activity. One contained lysine vasopressin and the other α-MSH, poorly separated from CRF activity. Attention was first directed to the CRF activity accompanying α-MSH. In a preliminary report with insufficient detail for critical evaluation it was stated that the CRF, denoted α-CRF because of its association with α-MSH, could be separated from α-MSH by paper chromatography or by column chromatography on carboxymethylcellulose. It was thought to consist of a single peptide or a mixture of peptides containing all the amino acids of α-MSH plus threonine, alanine, and leucine. It had ACTH activity of 0.1 unit/mg., vasopressin activity of 0.1 to 0.2 unit/mg. and about 1/10 to 1/15 the MSH activity of α-MSH. It was stated, without documentation, to be active *in vivo* and *in vitro* at dose levels of 0.5 to 2 μg. The material studied previously (194), called β-CRF, differed from α-CRF by the presence of cysteine, a 50 to 100-fold higher potency, and the absence of ACTH activity. Because of its higher potency, β-CRF obtained by countercurrent distribution was restudied (196). A somewhat improved but still unsatisfactory separation from lysine vasopressin was obtained by differential paper chromatography. A differentiation between the two substances was claimed on the basis of the concentration of CRF activity in the descending limb of the nitrogen curve, and biological activity *in vitro* and *in vivo* at 0.05 to 0.1 μg., in contradistinction to lysine vasopressin, which requires much larger doses. The task of clear separation of the two substances, however, still lies ahead.

Porter & Jones (158) observed that intravenous injection of plasma collected from the sella turcica of the dog following removal of the pituitary stimulated ACTH secretion in rats pretreated with cortisol. After fractionation of the plasma by the alcohol procedure (198), the activity was restricted to Fraction II + III (199); in the zinc precipitation method (200) it was found in Fraction III_0 (201). The latter fraction was separated into three zones by starch-column electrophoresis: the middle zone contained the activity (202). Under special conditions, the activity was dialyzable, suggesting dissociation from a carrier protein. Contamination

with ACTH, vasopressin, norepinephrine, epinephrine, histamine, and serotonin was minimal or undetectable, and systemic plasma was inactive. Rumsfeld & Porter have also reported (203) the fractionation of an acetone extract of bovine hypothalamus. Material obtained from the aqueous phase after partition of the acetone extract between ether and 0.9 per cent NaCl, containing no detectable ACTH activity, was fractionated first by ion exchange chromatography then by countercurrent distribution, resulting in the isolation of two active fractions, distinct from lysine vasopressin by paper chromatography. However, the minimum effective dose level was not stated.

Royce & Sayers (204) prepared an extract of acetone-dried and defatted calf median eminence and pituitary stalk tissue by refluxing in glacial acetic acid. The activity was precipitated with acetone and ether, extracted with 0.01 *M* ammonium acetate buffer at pH 5.8, and applied to a column of carboxymethylcellulose. In the median eminence-lesioned rat, marked hypothalamic hormone activity, substantially free of ACTH and vasopressin, was consistently found in one set of eluates at a dose level of 5 μg., equivalent to one-third of a median eminence-stalk section. In an earlier study (156) the initial extract was prepared with 0.2 *M* acetic acid and much of the ACTH was removed by treatment with oxycellulose. This relatively crude extract was active in the lesioned rat at a dose equivalent to as little as one-fortieth of a median eminence, suggesting that a considerable loss of activity was associated with the purification described above. McCann & Haberland (157) prepared and tested similar crude extracts from bovine and rat stalk-median eminence tissue with results in essential agreement with Royce & Sayers (156). McCann & Haberland concluded that the presence of a hypothalamic hormone had been demonstrated in the crude extracts, since the amounts of contaminating ACTH and vasopressin were insufficient to account for the activity in rats with median eminence lesions.

Leeman & Voelkel (205) homogenized fresh calf hypothalmus with 0.1 N HCl, lyophilized the supernate and re-extracted the product with distilled water in the cold. The extract reduced the adrenal ascorbic acid in anesthetized intact rats, contained little ACTH and vasopressin, and the effect was not inhibited by morphine, indicating that the extract contained HSF-ACTH. Further steps in the purification of the extract have not yet been reported.

Vasopressin as the hypothalamic secretory factor for ACTH.—Since several if not all of the known biologically active constituents of the hypothalamus [epinephrine, norepinephrine, acetylcholine, serotonin, histamine, substance P, and γ-aminobutyric acid (206)] can stimulate secretion of ACTH if injected in adequate amounts, the possibility that one or another of them is the postulated hypothalamic secretory factor for ACTH has been considered. The case for vasopressin has been forcefully presented by Rothballer (207), McCann (171), and a number of others. Intravenously

injected vasopressin, both natural and synthetic, is active in all the *in vivo* test systems described above for the assay of hypothalamic hormone; it is also active in the method in which halved pituitaries are incubated *in vitro*. Morphine inhibits the effect of vasopressin in the intact rat, but the inhibitory effect is surmounted by a fourfold increase in dose (178). Evidence against vasopressin as the secretory factor for ACTH was reviewed by Ganong & Forsham (150). One of the objections raised, the rather large doses required for positive effects, is true only for vasopressin administered systemically. Kwaan & Bartelstone (208) have shown that as little as two milliunits of synthetic arginine vasopressin injected into the third ventricle of the intact dog produce a marked increase in the adrenal venous 17-hydroxy-corticosteroids; the same dose of synthetic oxytocin had no effect. However, there is still a sound basis for the current consensus of opinion that vasopressin is not the hypothalamic secretory factor for ACTH, chemical evidence in the separation of active fractions of hypothalamic and posterior pituitary extracts from vasopressin by several groups of investigators, as well as the dissociation of the ACTH-secretory from the antidiuretic effects of vasopressin in physiological experiments (150).

Other Topics

Biological assay of ACTH in blood.—Methods of biological assay for ACTH in plasma, their limitations and applications, particularly in the assay of human plasma, have been reviewed by Nelson (209) and Munson (210). The concentration of ACTH in normal human plasma is so low in relation to the dose requirement of currently available assay methods that concentrates of large volumes of plasma, prepared by as yet unperfected chemical procedures, must be administered in order to produce a minimum effect. However, rough quantitation of the elevated concentrations in the plasma of patients with Addison's disease or after bilateral adrenalectomy is possible. Several promising new assay methods, still under development, that require smaller amounts of ACTH were also described. The stability of ACTH in human and dog blood and plasma was studied by Meakin *et al.* (211, 212). Activity was lost rapidly over a period of several hours, but it was stable for several months if stored frozen. The inactivating system in plasma was destroyed by heat, confirming Pincus *et al.* (213), and was antagonized by L-cysteine. McFarland *et al.* (214) reported values much higher for ACTH in cavernous sinus blood (technique for collection described) of conscious sheep after electric shock than in peripheral blood which was collected simultaneously, confirming the results of Ganong & Hume (215) in the dog, but the unusually high values reported for peripheral blood raise some doubt about the reliability of the assays. Persky *et al.* (216) assayed extracts of blood from anxious patients and normal subjects for adrenal-stimulating factors in hypophysectomized rats. By the classic adrenal ascorbic acid method, ACTH was detected ($>$ 1.5 milliunit/100 ml.) in two of 11 patients and in none of the 15 controls. The adrenal

weight-maintenance factor [reviewed in (210)] was detected in eight of the patients and in only one of the controls.

Mechanism of action of ACTH on adrenal cortex.—The work of Haynes and co-workers, recently reviewed by Haynes *et al.* (217), Sutherland & Rall (218), and by Tepperman & Tepperman (219), has provided a rational biochemical basis for the effect of ACTH on the biosynthesis of the cortical hormones. ACTH was found to stimulate the accumulation of adenosine-3′,5′-monophosphate and the activation of phosphorylase in adrenal slices. The cyclic adenosine monophosphate mediates the effect of ACTH on phosphorylase in the adrenal cortex just as it mediates the effect of epinephrine and glucagon on phosphorylase in the liver. However, ACTH has no such effect on liver or muscle, and glucagon and epinephrine are ineffective in the adrenal cortex. The hypothesis outlined by Haynes states that ACTH, by increasing adenosine-3′, 5′-monophosphate and, in turn, active phosphorylase in the adrenal cortex, stimulates the breakdown of glycogen to glucose-1-phosphate, thence to glucose-6-phosphate. The further metabolism of glucose-6-phosphate by the dehydrogenase system leads to an increased supply of reduced TPN, known to be important in hydroxylating reactions in the biosynthesis of the adrenal cortical steroids. However, the predominant action of ACTH on steroid biosynthesis had previously been localized at the stage of conversion of cholesterol to progesterone, rather than in the later hydroxylations. The work of Halkerston *et al.* (220), therefore, showing the requirement of reduced TPN for sidechain cleavage of cholesterol was particularly important in establishing the pertinence of Haynes' hypothesis.

It is not yet clear how relevant the effect of ACTH on the formation of cyclic adenosine monophosphate is for the many other actions of the hormone on the adrenal cortex, including secretion of steroids and ascorbic acid and the stimulation of increased size of the gland. Other interesting data and speculations on the mechanism of action of ACTH on the adrenal cortex have been presented by Hayano *et al.* (221) and Hechter & Lester (222).

Inhibitors of adrenal function.—In 1955 Hertz *et al.* (223) reviewed the remarkable spectrum of biological effects possessed by amphenone B [1, 2-bis-(p-amino-phenyl)-2-methyl-propanone-1], including progestational, antithyroid, and anesthetic actions, and in the rat the production of marked adrenal hypertrophy with deposition of increased amounts of cholesterol. It is now clear from studies in the dog and in man that amphenone B interferes with the biosynthesis of the adrenal cortical steroids; adrenal hypertrophy results from increased ACTH secretion in response to the declining level of circulating glucocorticoids. Tests on a series of 61 analogues of amphenone B prepared by Bencze & Allen (224) showed that it was possible to vary the potency and quantitative pattern of activities. Sixteen of the active analogues were examined in some detail by Chart & Sheppard (225), for the effect on adrenal and thyroid weight, natriuresis-

and hypothermia in rats, the adrenal venous cortical steroids in the dog, and the action on *in vitro* corticosteroid production by rat adrenal slices. A pyridine analogue, methbipyrapone [1,2-bis-(3-pyridyl)-2-methyl-1-propanone] (SU-4885), relative to amphenone B, was more active as an inhibitor of adrenal function, possessed a narrower range of biological activities, and was less toxic. Primarily it inhibits the 11β-hydroxylation of steroids, thus eliminating or drastically reducing the biosynthesis and secretion of cortisol, corticosterone, and aldosterone. Chronic administration, however, may eventually lead to sodium retention, through compensatory increase in ACTH secretion and a marked overproduction of the active 11-deoxy mineralocorticoids, Substance S (11-deoxycortisol) and cortexone (deoxycorticosterone). In clinical use the abnormal steroid production may be avoided by simultaneous administration of a potent glucocorticoid to inhibit excessive ACTH secretion. SU-4885 is also useful in the differential diagnosis of pituitary-adrenal disorders, since if the anterior pituitary ACTH-secreting mechanism is capable of functioning normally, the administration of the drug is followed by a rise in 11-deoxy cortical steroid levels and by natriuresis. Additional details and an excellent review are included in the paper by Chart & Sheppard (225).

Aldosterone antagonists.—The native hormones, progesterone and testosterone, are weakly natriuretic and also show some antagonism to the action of mineralocorticoids. However, a series of synthetic steroidal spirolactones synthesized and studied by Cella & Kagawa (226) proved to be more highly effective aldosterone antagonists, active in man (227) as well as in experimental animals. Except for a very weak progestational effect, they are apparently without other significant biological actions. The effective daily dose in man of the original compound studied, 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl)-propionic acid-7-lactone (SC-5233), was rather high—400 mg. intramuscularly or several grams orally. However, the 17-nor and doubly and triply unsaturated analogues were more potent. Spironolactone, the 7-acetyl thio derivative of the original compound, is available for clinical use and is effective in a daily dose of 100 mg. orally.

The spirolactones antagonize the retention of sodium, chloride, and water and the excretion of potassium induced by mineralocorticoids, but are without detectable renal effects in the absence of an excess of these steroids. The evidence that the mechanism of action of the spirolactones is a direct antagonism of the renal tubular action of aldosterone and other mineralocorticoids is as follows. They have little or no effect on urinary electrolytes in adrenalectomized animals or patients with adrenal insufficiency in the absence of mineralocorticoid therapy. The electrolyte excretion pattern produced by spirolactones in patients with intact adrenals is similar to that following abrupt withdrawal of aldosterone. The spirolactones do not act by interfering with the biosynthesis or secretion of aldosterone or other adrenal cortical steroids, since they have been shown not to cause significant changes in the rate of secretion of aldosterone, or in the

amounts of aldosterone, tetrahydro-aldosterone, 17-hydroxy-corticoids, or 17-ketosteroids in the urine. Therapeutically, the spirolactones used in conjunction with more conventional diuretic agents tend to combat the tendency of these drugs to induce kaliuresis through increased aldosterone secretion. Aldosterone antagonists have been reviewed recently by Beyer (228), Gaunt *et al.* (229), Kagawa (230), and Coppage & Liddle (231), and a symposium on the subject has been published in book form (232).

Chemical structure and biological activity of insulin.—A review of the chemistry of insulin by Sanger (233) includes a section on the relation between biological activity and chemical structure. That free amino groups are not essential is indicated by the absence of effect of acetylation on activity. On the other hand, since esterification leads to loss of activity, one or more of the six carboxyl groups must be essential. It is not the C-terminal alanyl carboxyl on the phenylalanyl (B) chain, since enzymatic removal of the alanine does not affect activity. Furthermore, C-terminal alanine (B chain) in bovine, pig, sheep, horse, and whale insulin is replaced by serine in rabbit insulin and by threonine in human insulin without detectable difference in activity. C-terminal asparagine (A chain), however, is essential as shown by loss of activity following its enzymatic removal. The importance of the phenolic hydroxyl groups of tyrosine was demonstrated by loss of activity following iodination. Species differences in the amino acid sequence inside the disulfide ring of the A chain—Thr · Ser · Ileu (pig, whale, rabbit, human); Ala · Ser · Val (bovine); Ala · Gly · Val (sheep); Thr · Gly · Ileu (horse)—without change in biological activity demonstrate the unimportance of the exact structure of this part of the molecule. It was concluded from the results of studies on the effect of leucine aminopeptidase that the first six (N-terminal) residues of the B chain are not essential for activity. After rapid splitting off of C-terminal alanine, trypsin slowly splits the bond between arginine and glycine in the B chain, yielding a heptapeptide, which is biologically inactive, and the residual molecule (DHA insulin). DHA insulin retained about 15 per cent of the activity of intact insulin, indicating that the absent fragment, although not essential, contributed materially to the activity. Recent work has suggested some modification of Sanger's statement that the disulfide bridges must remain intact for activity. After splitting the interchain disulfide bonds of insulin with sulfite, Cecil & Leoning (234) partially resolved the product into A and B chains. A sample of A chain containing approximately 20 per cent of B chain as impurity was tested for insulin-like activity in the isolated perfused rat heart (penetration of L-arabinose) (235) by Fisher & Zachariah (236) and found to be active, although less than 10 per cent as active as intact insulin. Whether or not unchanged insulin had been eliminated from the sample tested was not clear. Langdon (237) cleaved the disulfide bonds of insulin with thioglycollate or *n*-propyl mercaptan and separated the two resulting peptide chains. The A chain was biologically inactive. The phenylalanyl chain, as the phenylmercuric derivative, was obtained in crys-

talline, electrophoretically homogeneous form. It was active in stimulating the metabolism of rat adipose tissue in two *in vitro* test systems (238, 239). The amounts tested, however, were 200 times or more than the equivalent amount of insulin required for similar effects, and the test systems are not specific for insulin. Therefore, although care was taken to exclude the possibility that unchanged insulin contaminated the test sample, some doubt remains as to the significance of the results.

Orally active hypoglycemic agents.—A detailed critical review by Duncan & Baird on the pharmacology of the orally active hypoglycemic agents, with emphasis on the current status of knowledge of mechanisms of action, was published in March, 1960 (240). There are shorter reviews by Ashmore (241) on mechanisms of action and by Dunlop (242). The paper by Mahler (243) is concerned principally with the relation between chemical structure and biological activity.

Relation of chemical structure to biological activity of analogues of thyroxine.—The approximate relative potencies of 55 active analogues of thyroxine in three types of biological assay, oxygen consumption, growth and differentiation, and prevention of thiouracil-induced goiter, have been tabulated and evaluated by Pitt-Rivers & Tata; 70 inactive analogues were also listed (244). The data were obtained from the reports of many different investigators; it was hardly feasible to express the results in the language of biostatistics. Nevertheless, certain conservative generalizations were possible. The basic diphenyl ether structure is essential but not in itself adequate for activity. At least two halogen substituents are necessary; the highest activity being associated wtih iodine substitution, bromine less, and chlorine still less. Trihalogenated compounds substituted in the 3, 5, and 3′-positions are almost invariably more active than the corresponding tetrahalogenated compounds. The position of the halogen substituents, the length of the side chain, the phenolic group and its degree of ionization, and the presence of a free carboxyl group in the side chain are additional important structural features.

The data reported by Stasilli *et al.* (245) concerning the relative potency in the goiter-prevention and calorigenic assay methods of 45 compounds related chemically to thyroxine were included in the summary by Pitt-Rivers & Tata. Subsequently, the same compounds were tested by Money *et al.* (246) for their inhibitory effect on collection of ^{131}I in the rat thyroid. Twenty-seven compounds were tested by Kumaoka *et al.* (247) for their effect in depressing the growth of a thyroid-stimulating hormone-secreting pituitary tumor in mice; thyroxine and triiodothyronine were the most potent compounds tested, and in general there was a high correlation with results obtained in the goiter-prevention assay. The lactic acid analogues of 3, 5-diiodothyronine, 3, 5, 3′-triiodothyronine and thyroxine were found to be less potent in the goiter-prevention assay than the corresponding parent compounds and acetic and propionic analogues (248). All analogues of thyroxine tested depress serum cholesterol at lower dose levels

than are required to increase oxygen consumption *in vivo* in the rat and in man (249, 250) or by excised tissues after previous treatment of the whole animal (249). However, the recent discovery that androsterone depresses serum cholesterol (24) should turn the search for compounds with this property in a new direction.

Pittman & Barker (251, 252) discovered that the weakly thyromimetic compounds, 3, 3′, 5′-DL-triiodothyronine and 3, 3′-DL-diiodothyronine, and their propionic acid analogues, in doses 100 times that of thyroxine, completely and reversibly antagonized the calorigenic effect of thyroxine in thyroidectomized rats. At the same dose levels, the acetic acid analogues were less active. Previously known antagonists, reviewed by Selenkow & Asper (253), were much weaker, with effective doses 250 to 2000 times that of thyroxine. The limited supply of 3, 3′, 5′-triiodothyronine has precluded extensive tests in man. In three of four treated hypothyroid patients, the compound significantly depressed the basal metabolic rate. It was inactive in one euthyroid subject and in one patient with hyperthyroidism (254). At higher doses, however, it reduced the basal metabolic rate in one patient with Graves' disease (255). Jorgensen *et al.* (256) reported four alkyl-substituted analogues of thyroxine that antagonized the antigoitrogenic effect of thyroxine at dose levels 100 to 200 times that of thyroxine. In intact rats they did not antagonize the calorigenic effect of thyroxine; they were not tested in thyroidectomized rats.

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THE ACTION OF DRUGS ON THE SKIN[1]

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The skin is more accessible to observation and treatment than any other part of the body, but our knowledge of its pharmacology is rather limited. Investigators in many different fields have used the skin for their experiments because it happened to be convenient—not because they were particularly interested in it. Reports of much of this work are scattered and not easily accessible, so that it seems useful to review some of it for pharmacologists as well as dermatologists. This article aims to survey a part of the field in detail; in many sections, however, space will only permit brief indications of where information is to be found. Special emphasis will be given to recent work and to human pharmacology. The literature on abnormal skin is not covered.

Methods of Applying Drugs Locally

A drug whose effects on the skin are to be studied may be applied in various ways. It may be merely placed on the intact skin, it may be rubbed in, driven in by iontophoresis, pricked in, or injected intradermally or intraarterially. The advantages and disadvantages of these various methods will be briefly discussed.

Contact and inunction.—These provide more uniform application to larger areas of skin than is possible with other methods. The dose of drug that actually enters the skin can only be determined by fairly elaborate methods [e.g., see Blank *et al.* (1); Gemmell & Morrison (2, 3); Griesemer *et al.* (4); Malkinson (5); Schulze & Reiff (6)]. The trauma of inunction, although usually slight, may in some circumstances be considered a disadvantage. For example, in patients with atopic dermatitis the unaffected skin tends to respond to mechanical stimuli by blanching, and this blanching after inunction of an ointment may be wrongly attributed to a constituent of the ointment [Reed, Kierland & Code (7)].

Iontophoresis.—Only electrolytes can be applied by iontophoresis. Theoretically the effects of the anionic and cationic constituents of a drug could be studied separately, but this is unlikely to be important. It is difficult to gauge the dose that enters the body and even more difficult to determine what proportion is in the skin, though attempts have been made, e.g., by Ghys (8). The passage of electric current may interfere with the effect of the drug. Iontophoresis requires relatively large amounts of drug.

Puncture or scarification, and intradermal injection.—The major limitation of these procedures is that drug effects can only be produced in a

[1] The survey of the literature pertaining to this review was concluded in August, 1960.

small area. Intradermal injection permits more accurate dosage and requires much less material than does puncture, but the latter is less traumatic. Much of the fluid introduced by puncture or scarification probably does not penetrate to the dermis in the first instance and acts predominantly on the epidermis; fluid introduced by superficial intradermal injection tends to lie more deeply, in the region of the dermoepidermal junction. That there may be an important difference between the two routes is illustrated by Broadbent's observations (9) on histamine-induced pruritus. Squire (10) matched histamine weals produced by puncture and by intradermal injection. He estimated from the results that the volume he introduced by a single prick was approximately 3×10^{-6} ml.

The technique of intradermal injection is important. Fine needles (diameter 0.45 mm.) are most often used. Whether short-bevel or long-bevel needles have any advantage is not certain. For injection into the most superficial layers of the skin the needle is inserted almost parallel to the skin surface, with the bevel facing upwards; having entered the skin, the needle is then advanced toward the skin surface until the bevel can be seen through the intact skin. With the needle in this position the fluid is injected. Most intradermal injections are made using tuberculin syringes graduated to 0.01 ml. Unfortunately many such syringes leak under pressure; therefore it is advisable to test syringes that are to be used for accurate work [Guld & Rud (11)]. Recently, however, a new type of syringe has become available in which the plunger is fitted with a small rubber ring; this syringe does not leak when tested by Guld & Rud's method (11a). Some other sources of error in the technique of intradermal injection, e.g., variation in depth, have been studied by Palmer & Edwards (12) and Guld (13) in connection with BCG vaccination and tuberculin testing.

Intraarterial injection or infusion.—These methods allow distribution of a drug to the skin of the distal part of a limb and do not entail any interference with the skin. However, the distribution of the drug to the skin is liable to be uneven because of differences in blood supply between different areas and tissues and because the injected solution may not mix uniformly with the blood flowing along the artery. A drug such as acetylcholine may be largely destroyed in the blood while it is passing from the site of injection to the skin vessels. These and other snags are discussed more fully by Duff *et al.* (14).

Skin Blood Flow

Recent reviews covering particular aspects of the cutaneous circulation are those by Furchgott on the pharmacology of vascular smooth muscle (15), and by Paton on vasodilator drugs (16). This section attempts to supplement these but excludes substances whose main action is indirect, i.e., at sites remote from the skin.

Most of the numerous methods that have been used to study skin circulation are described in *Methods in Medical Research* (17). Others are

those of Hensel & Bender (18), Bárány (19), Burch (20), and Fox & Wyatt (21). The multiplicity of methods is partly attributable to the difficulty of discriminating between the various parts of the skin circulation. There are important regional differences (e.g., in man between hand, forearm, and face) so that results obtained in one region do not necessarily apply in another. In addition, within each region the responses of different types of vessel often differ. Routine pharmacological studies of new compounds in animals frequently include observations on limb blood flow, but such data include skin and muscle blood flows and can give no reliable information about the skin.

Much of the quantitative work with drugs on skin blood flow has been done in man, using venous occlusion plethysmography of the hand. The hand contains about twice as much skin and subcutaneous tissue by volume as it does muscle [Abramson & Ferris (22)], so that the greater part of the hand blood flow is generally assumed to represent skin flow. The forearm contains several times more muscle than skin (22); the forearm blood flow is, therefore, an unreliable indicator of the blood flow in the forearm skin.

Sympathomimetic substances.—Epinephrine and norepinephrine have a powerful constrictor action on the vessels of the hand and foot [Barcroft & Swan (23)]. Glover *et al.* (24) have recently shown that both the prevenous resistance vessels and the capacity vessels, presumably mainly venous, are affected. Epinephrine is somewhat more potent than norepinephrine in reducing hand blood flow; this also seems to be true for skin vessels in other parts of the body (23). Isoprenaline (isoproterenol) infused into the brachial artery causes slight vasodilatation in the hand, or no change [Cobbold, Ginsburg & Paton (25)]; in the dog it increases the flow in the saphenous artery, which supplies only skin [Walters *et al.* (26)].

Adrenergic blocking agents.—Ergot alkaloids have a powerful direct constrictor action on skin vessels. For example, the dihydrogenated derivatives of the ergotoxine group (Hydergine) cause vasodilatation in the hand and foot by two distinct mechanisms, namely a local adrenergic blocking action and a central inhibition of vasoconstrictor tone [Barcroft, Konzett & Swan (27)]. Tolazoline and phentolamine have a direct vasodilator action in the skin as well as adrenergic blocking activity (28). Given by intraarterial infusion, phenoxybenzamine, which has a different mechanism of action, greatly reduces the constrictor response to epinephrine, norepinephrine, and to sympathetic stimuli; it also has a direct vasodilator action of its own [Duff & Ginsburg (29); Ginsburg & Duff (30)]. Bretylium tosylate is a different type of drug with a selective action on adrenergic nerves. In the perfused isolated rabbit ear it prevents the vasoconstriction caused by stimulation of sympathetic fibers but increases the vasoconstrictor effects of epinephrine and norepinephrine [Boura *et al.* (31)]. In man, intraarterial infusion produces moderate vasoconstriction [Blair *et al.* (32)], which may be followed by vasodilatation (31, 32). Reflex vasoconstrictor responses in the hand are reduced or abolished. Guanethidine appears to

have a similar mechanism of action [Maxwell, Mull & Plummer (33)]. The action of chlorpromazine has also been studied. This drug has a direct vasodilator action in the hand; in addition, it antagonizes norepinephrine specifically, but clear-cut antagonism to epinephrine could not be demonstrated [Ginsburg & Duff (34); Duff & Ginsburg (35)].

Hydrocortisone.—By itself, hydrocortisone infused intraarterially (total dose 0.6 to 1.2 mg.) does not affect hand blood flow, but epinephrine given immediately afterward causes a greater decrease in flow than in an untreated hand [Ginsburg & Duff (36)]. This potentiation does not occur with norepinephrine, and is difficult to explain.

Histamine, acetylcholine, and their antagonists.—Histamine and also acetylcholine cause a great increase in both the hand and the forearm blood flow when infused intraarterially [Duff *et al.* (14)]. Another interesting observation with a histamine analogue, 3-β-aminoethyl-1,2,4-triazole, has been made by Naftchi *et al.* (37). Blood pressure, mouth temperature, and finger blood flow were recorded before and at intervals after an oral dose of this substance. The drug caused flushing of the face and chest, and the fingers also showed vasodilatation; all the subjects felt chilly, and mouth temperatures fell. A striking fall in finger blood flow occurred in all subjects. Similar effects were observed after subcutaneous injection of histamine itself. The authors suggest that the heat loss following general capillary and venular vasodilatation caused reflex constriction of the digital arteriovenous anastomoses, thus decreasing the finger blood flow. A direct constrictor action of histamine or its analogue seems a less likely explanation.

Intraarterial atropine in doses that block the vasodilator effect of acetylcholine does not affect the hand blood flow [Gaskell (38); Allwood & Ginsburg (39)]. The flushing of the skin, particularly of the face, which often occurs after the systemic administration of atropine and similar drugs, does not appear to have been investigated, and its mechanism is unknown.

Serotonin (5-hydroxytryptamine).—The responses of the skin vessels to serotonin are complex. Roddie, Shepherd & Whelan (40) found that intraarterial infusion causes a decrease in hand blood flow accompanied by flushing of the skin and an increase in the volume of the part. This indicates constriction of the vessels mainly responsible for resistance to flow and dilatation of those responsible for the color of the skin. Reid (41) had previously shown that serotonin injected near a vein causes constriction. The ingenious observations of Glover *et al.* (24) have demonstrated that the low-pressure capacity vessels in the hand become less distensible when an intraarterial infusion of serotonin is given. These changes explain how the characteristic cyanotic flush is brought about. Intradermal doses of serotonin larger than those required to elicit these vascular reactions cause wealing; indirect evidence suggests that this may be caused by liberation of histamine [Möller & Rorsman (42); Demis, Davis & Lawler (43)].

It is not certain whether serotonin plays any part in the characteristic

attacks of flushing that occur in some patients with metastasizing carcinoid tumors. These tumors often contain large amounts of serotonin, and an abnormally high concentration may be present in the blood. Discussion of this problem [e.g., by Snow *et al.* (44); Smith *et al.* (45)] suggests that some substance other than serotonin may be responsible for the flushing. The discovery by Schneckloth *et al.* (46) and Peart *et al.* (47) that intravenous norepinephrine or epinephrine will provoke typical flushes in carcinoid patients should speed the search for such a substance.

Adenosine triphosphate (ATP).—This substance is of special interest because there is evidence that it may be the transmitter released from sensory nerve endings causing antidromic vasodilatation [Holton (48)]. In man, ATP is as powerful a vasodilator as histamine and acetylcholine [Duff, Patterson & Shepherd (49)]. Holton & Holton (50) compared the vasodilator activity of ATP with that of various substances related to it, using their denervated rabbit-ear preparation. The activities of adenosine, and of its di- and monophosphates, were of the same order as that of ATP; the other substances tested were much less active.

Peptides.—Vasopressin has long been known as a powerful constrictor of capillaries and arterioles [Goodman & Gilman (51); Kitchin (52)]. The circulatory effects of oxytocin in man have recently been investigated by Kitchin, Lloyd & Pickford (53). Given intravenously or intraarterially it causes considerable vasodilatation in the hand. Administration of one part of vasopressin together with 20 parts of oxytocin prevents this vasodilatation. Valyl^3-oxytocin is about 1.5 to two times as active a vasodilator as the synthetic oxytocin, Syntocinon [Kitchin, Konzett & Pickford (54)], whereas it has about four times the oxytocic effect of Syntocinon on the human uterus [Smyth (55)].

Bock, Krecke & Kuhn (56) have studied the effects of intravenous and intraarterial administration of synthetic hypertensin II in man, using Hensel's heated thermocouple method to measure skin and muscle blood flow. Vasoconstriction occurred in both vascular beds but was more intense and lasted longer in the skin. The vasoconstriction in the skin was greater, while in muscle it was much less, than that produced by norepinephrine.

Bradykinin, which has recently become available in pure form, is a potent vasodilator in man and is about as active as histamine by intraarterial injection [Fox *et al.* (57)].

Rubefacients.—The term rubefacient embraces a heterogeneous collection of substances intended for local application to the skin. Many have a long history in therapeutics and have been little investigated. A number of these old-established rubefacients, such as mustard oil and cantharidin, cause inflammation, and this probably explains why they redden the skin. Some substances, e.g., capsaicin and camphor, stimulate certain sensory nerve endings; this may have something to do with their rubefacient activity. Histamine and histamine liberators such as ethylmorphine (Dionine)

constitute another group; these are very effective when applied by electrophoresis. The last and most recently introduced group consists of various esters of nicotinic acid.

Toh, Lee & Kiang (58) have studied the pharmacology of capsaicin, the main active principle of various species of *Capsicum,* and of several of its analogues, derivatives of vanillylamine. None of these substances had any direct action on the blood vessels of the perfused rabbit ear. This work has recently been extended by Crismon *et al.* (59) who found that inunction of 2 gm. of a water-miscible cream containing 0.1 per cent capsaicin into the skin of the forearm produced no definite increase in the forearm blood flow. After similar application of a cream containing 2.5 per cent benzyl nicotinate, the forearm blood flow was almost doubled. Reddening and a local sensation of warmth or burning were produced by both creams, the sensation being more marked and sustained after capsaicin and the reddening more marked after benzyl nicotinate. Block of the cutaneous nerves to the forearm skin before inunction of the nicotinic acid ester did not affect the vasodilatation, showing that the response does not depend on an intact nerve supply. Doerr & Heite (60) had earlier investigated a cream containing both 2.5 per cent benzyl nicotinate and 0.1 per cent capsaicin. The skin blood flow was measured calorimetrically and skin color photometrically. A substantial increase in blood flow occurred in all 50 subjects. In many the skin became redder, but the correlation between blood flow and color was low. It is interesting that the mean values for patients with atropic eczema did not differ significantly from those for normal subjects, because in atopic skin the superficial minute vessels are relatively unresponsive to dilator stimuli [for references see also Clendenning *et al.* (61)].

A different approach is that of Fulton *et al.* (62), who have studied the microcirculation in the cheek pouch of the hamster using a cinematographic technique. Four different esters of nicotinic acid, applied topically to vessels from a micropipette consistently produced vasodilatation in arterioles and precapillary arteriolar sphincters, but not in other vessels. The reaction was prompt and uniform, even at some distance, suggesting that the perivascular nerve plexus may be involved. This method may supply useful information about the action of rubefacients, though the vessels in the hamster's cheek pouch probably differ from those in human skin.

The nicotinic acid esters present several other interesting problems. Given by mouth or injected, nicotinic acid itself produces vasodilatation which is most prominent in the blush area and seems to be much less elsewhere. How is this vasodilator effect related to that of the esters? Some of the esters, thurfyl nicotinate (Trafuril, tetrahydrofurfuryl nicotinic acid) and benzyl nicotinate (63), frequently cause edema at the site of application. Pretreatment with antihistaminic drugs, either locally or systemically, appears to have no effect on this edema formation, suggesting that it is not caused by histamine release, but this point is far from settled.

In Great Britain alone at least eight different esters of nicotinic acid

are on sale in commercial rubefacient preparations, most of which contain other agents as well [Martindale (64)], and new ones continue to appear [e.g., Mombaerts (65)]. An objective comparison of these compounds is overdue.

Vascular Permeability

There is no evidence suggesting that the permeability of arterioles or of veins is important, and the subject has been little studied. Veins are permeable to some substances, for in man intravenous injection of histamine or of pethidine (meperidine), a histamine liberator, may cause linear wealing to appear in the skin overlying the vein. This is particularly marked when the flow of blood is obstructed. Mepyramine can apparently also pass through the vein wall [Finer & Partington (66)].

Substances that affect capillary permeability have recently been reviewed by Spector (67). Capillaries in the skin are normally impermeable to protein. Increased capillary permeability is demonstrated when plasma protein appears in the tissue fluid. It is harder to establish that foreign protein injected subcutaneously appears in the plasma before it appears in the thoracic duct lymph. A method that has been widely used in animals depends on the leakage of protein-bound dye, such as pontamine blue or Evans blue, from the plasma into the skin. The dye is injected intravenously, and the test substances intradermally. The size and intensity of the color of the lesion are used as a measure of capillary permeability. A few investigators have used Evans blue in this way in man [Demis *et al.* (45); Stewart & Bliss (68); Herxheimer & Schachter (69)]. Its only disadvantage seems to be that in some subjects the skin may show a faint greenish-grey tint for a few days. Sodium fluorescein has also been used [e.g., Möller & Rorsman (70), who give further references], but since it is incompletely bound to plasma protein its appearance in the skin does not necessarily indicate increased capillary permeability.

Miles & Miles (71) made extensive use of the dye method in the guinea pig, and their paper contains many important observations. With graded doses of histamine, the histamine liberator 48/80, and leukotaxine injected in a constant volume, the diameter of the lesion was proportional to the logarithmic dose of the drug. The measurements obtained when the dose was kept constant and the volume varied were used to measure the affinity of skin tissue for the substance injected. Histamine has a relatively low, and 48/80 a relatively high "affinity" for skin tissue. This partly explains why high doses of 48/80 produce smaller and more intensely blue lesions than do high doses of histamine. The same authors also studied the time course of the histamine effect, i.e., the rate of "fixation" of histamine in the tissue, the duration of increased permeability, and the duration of the immunity or refractoriness at injection sites to further doses of histamine or 48/80.

Various factors that influence the reactivity of guinea pig skin to his-

tamine were also examined (71). Important regional differences exist between the reactivity in the ear, the back or flank, and the abdomen near the midline. The explanation of these differences is not certain, but the greater vascularity of the ear may have something to do with it. The ventral skin of the trunk is thinner than dorsal skin, and fluid entering it probably spreads more easily. In general, factors that decrease intravascular pressure diminish the reactivity to histamine. Examples of such factors are lowered environmental temperature (10°C.), general anesthesia, shock produced by sublethal doses of *Escherichia coli* endotoxin or other means, and pretreatment with posterior pituitary extract. Such diminution of histamine bluing by pretreatment with a vasoconstrictor agent is the basis of Lockett & Jarman's (72) assay of the capillary action of flavanoid compounds in mice. Presumably, the method does not discriminate between any arteriolar constriction that may occur and capillary constriction. A raised environmental temperature (37°C) also diminishes reactivity to histamine in the guinea pig (71). Lewis & Grant (73), discussing a similar phenomenon in man, suggested that it may be caused by more rapid removal of histamine from the injection site.

A difference between the guinea pig and man which remains unexplained is that, in man, increased permeability of the minute vessels of the skin leads to weal formation, whereas in the guinea pig a definite weal can seldom be seen or felt. The dog and the horse are like man in this respect (74); most of the other common laboratory animals are like the guinea pig. This property of human skin has made it possible to study capillary permeability in man without the use of dyes, but until such methods have been tested directly against one of the dye techniques, one cannot exclude the possibility that they may give different results, unlikely though this seems. This needs to be done.

Simple quantitative observations can be made by measuring the size of weals produced by injecting or puncturing the test substance into the skin. Since weals have depth as well as area it is logical to try to measure weal volume, but this has been attempted by only few investigators. Pfeiffer, Jenney & Williams (75) constructed a dermal plethysmograph for the purpose and made a few observations with it in man. Smith & Humphrey (76) and Humphrey (77) calculated the volume of local inflammatory edema in guinea pigs and rabbits from its depth and area. Many investigators have used weal area alone as a measure of effect [Squire (10); Bain, Hellier & Warin (78); Holti (79)]. Bain *et al.* (78) found that the weal area measured directly agreed closely with that calculated from the mean radius, as obtained from the average of the greatest diameter and the one at right angles to it. This approximation is reasonable when the difference between the two diameters is small, and offers a considerable saving of labor. A better approximation, however, which also holds when a weal is more elliptical in shape, is obtained by multiplying the product of the two diameters by $\pi/4$ [Engelhardt, Funk & Heite (80)].

Bain, Broadbent & Warin (81) found that the relationship between the

log of the dose of histamine and the weal area was rectilinear over a 300-fold range of dosage. This contrasts surprisingly with the findings of Miles & Miles (71) in the guinea pig that the diameter of the lesion is proportional to the log of the dose. They suggest that the difference may lie "in the modification of histamine spread or of weal diameter, by the copious exudation that occurs in man but not in the guinea pig." The contrast between the two types of response may, however, be more apparent than real, because in their assay Miles & Miles used only four doses, covering a thirty-fold range. Over such a restricted range, the line relating log of the dose to lesion diameter might be so slightly curved that it would appear as straight as the line relating log of the dose to area. The data of Bhoola, Calle & Schachter (82) lend some support to such an explanation, for these workers found the log dose/lesion-diameter line for histamine in the guinea pig to be curved. When their data are plotted using the square of the diameter instead of the diameter, the line appears straight.

In man, as in the guinea pig, many factors affect the local response to histamine. They are briefly reviewed by Engelhardt *et al.* (80), and only a few will be mentioned here. Wealing in response to other substances is also likely to be affected by these factors. Stüttgen *et al.* (83) compared 10 different sites in 34 subjects and found definite regional differences. The largest weals in response to a particular dose occurred on the anterior thigh, the smallest on the extensor surface of the forearm. In adults higher doses of histamine are required to elicit wealing with increasing age [Tuft *et al.* (84)]. Skin treated with x-rays [Shaffer (85)], histamine iontophoresis, or ultraviolet radiation [Holti (79)] shows a diminished wealing response, as well as impaired vasoconstrictor tone in the minute vessels. Such effects may persist for many months (79).

Information that has a bearing on capillary permeability, but is less directly related to it, is obtained from observations on the time taken for injection weals to disappear. The weal disappearance time is influenced not only by capillary blood flow and permeability, but also by the degree of "spreading" of fluid in the tissue, by the rate of reabsorption of fluid in the venous limb of the capillary, and by the patency of the lymphatics. Heite and his co-workers (86 to 89) have studied some of these problems by injecting solutions of fluorescein into the skin of the guinea pig and of man, and recording the weal disappearance time concurrently with the disappearance time of fluorescein staining. The combination of these two measures has helped greatly in the interpretation of the results. After intravenous calcium gluconate, for example, the injection weal disappeared more rapidly, while fluorescein staining persisted longer than normal, suggesting that reabsorption of fluid into the capillary was increased and that "rinsing" of the tissue spaces by capillary filtrate decreased (89). This is evidence against the traditional view that calcium decreases permeability along the whole length of the capillary, for if that were true the weal should have persisted longer than normal.

This is not the place for discussion of the complex problems of fluid

exchange between capillaries, tissue spaces, and lymphatics. They are fully dealt with in the excellent book on the lymphatic circulation by Rusznyák, Földi & Szabó (90) which has just appeared in an English version (91). This also contains much information on the effects and possible role of hyaluronidase.

Mention must now be made of some recent work on histamine, histamine release, mast cells and related topics, and peptides. Important studies on histamine in Kahlson's laboratory (92) have established that there is no correlation between the rate of histamine production in a tissue and its histamine content. The differences in histamine metabolism in the fetus and the adult are particularly striking [see also West (93)]. Another series of experiments has shown that healing of skin wounds in the rat can be accelerated or slowed respectively by increasing or decreasing the histamine-forming capacity [Kahlson *et al.* (94)]. Archer's observation (95) that eosinophil leukocytes in the skin of the horse respond chemotactically to histamine may possibly be relevant here.

Histamine liberators and the mechanism by which they act have been discussed by Paton (96) and Uvnäs (97), the physiopathological role of histamine and its liberation in man by Lecomte (98). Riley's monograph on mast cells (99) and West's review of tissue mast cells and tissue amines (100) both include material on histamine, serotonin, and their release. The mechanism of liberation of histamine and of a fat-soluble smooth muscle stimulating substance (SRS) in relation to mast cells in various tissues has been extensively studied in Uvnäs's laboratory (101). Parratt & West (102) have found that thyroxine greatly increases the sensitivity of the rat to histamine, serotonin, and substances which liberate them. Thyroxine appears to inhibit intestinal histaminase so that released histamine accumulates.

Halpern *et al.* (103) have compared the ability of promethazine, chlorpromazine, mepyramine, and *N,N*-diethyl-D-lysergamide to inhibit edema induced in the rat by histamine, serotonin, dextran, and histamine liberators 1935L and 48/80. Their effect on anaphylaxis in the guinea pig was also examined. Other workers have studied the inhibition in the rat of serotonin-induced edema by lysergic acid derivatives and antihistaminic drugs [Doepfner & Cerletti (104)], and of dextran-induced edema by various phenothiazine derivatives [Kató & Gözsy (105)], and by 2-deoxyglucose [Goth (106)].

The work of Bhoola, Calle & Schachter (82) has strengthened the suggestion that the release of kallidin and similar substances (e.g., bradykinin and pain-producing substance) from plasma by dilution, or by contact with glass, is a result of the activation of the enzyme kallikrein. The permeability-enhancing activity of the permeability globulins in fractionated plasma [Mill *et al.* (107)] and in inflammatory exudates [Spector (108)] may be attributable to kallikrein. Preliminary observations in man have shown that bradykinin, kallidin, and kallikrein have similar effects when

injected intradermally [Herxheimer & Schachter (69)]. The role of such substances in inflammation, pathological pain [Keele (109)], and certain forms of urticaria [Herxheimer (110)] is still obscure.

Sweating and Pilomotion

In 1955 Randall & Kimura (111) reviewed the pharmacology of sweating; other reviews have only covered limited aspects of the subject. Weiner & Hellmann (112), in their admirable review on the sweat glands, have discussed pharmacological observations bearing on the regulation of sweat secretion; in an article on the autonomic innervation of the skin, Herxheimer (113) has dealt with axon-reflex sweating and other axon reflexes in the skin.

Substances that inhibit sweating when applied locally to the skin have been reviewed by Sulzberger & Herrmann (114). These authors have also summarized the work on sweat suppression by mepacrine. Klarmann (115) has discussed chemical and bacteriological aspects of antiperspirants and deodorants. Of the substances effective when given systemically, atropine-like drugs are the most important. Many such drugs are available. All of them have other anticholinergic effects which limit their therapeutic use. The most troublesome in this respect are probably scopolamine, atropine itself (not atropine methylbromide), and methantheline, but there seems little to choose between other drugs of this group [for further discussion see Herxheimer (116)].

Only two interesting recent observations can be mentioned here. Hankiss (117) has reported experiments on a patient with diabetes insipidus which indicate that antidiuretic hormone can reduce the elimination of water in the sweat. Previous workers [e.g., Pearcy *et al.* (118)] have not been able to detect such an effect in normal subjects, and further work will have to clarify the conditions in which this response can be demonstrated.

Bernstein & Sonnenschein (119) have found that 5 per cent tetraethylammonium chloride injected intradermally produces local sweating and piloerection, apparently by potentiating central tonic impulses to the sweat glands and pilomotor muscles. This curious effect deserves to be studied further with a greater range of concentrations than were used by these authors.

Sensory Receptors, Itching, Pain

Gray & Diamond (120) reviewed the pharmacological properties of sensory receptors in 1957. Since then the main advance has been in the investigation of nonmyelinated C fibers [e.g., by Douglas & Ritchie (121) and Iggo (122)]. The recording of electrical activity in single sensory fibers may be expected to give important information about the actions of pain-producing substances and of substances such as menthol and capsicin which evoke other types of sensation.

Chemical causes of pain and itch have been reviewed by Keele (109).

More recently a study group at the Ciba Foundation (123) discussed the nervous mechanisms of pain and itch, and also chemical factors. Two main groups of substances are known to cause itching on local application to the skin: histamine or histamine liberators and various proteolytic enzymes. Histamine produces more itching when injected intradermally in a buffered solution than in 0.9 per cent sodium chloride. Direct application of the buffered solution to scarified skin causes more prolonged itching and shorter-lasting pain than intradermal injection of the same solution [Broadbent (93)]. Several factors may account for these differences (9) but their relative importance is not yet clear.

Shelley & Arthur (124, 125) discovered that various proteolytic enzymes can cause itching and implied that this itching is associated with proteolytic activity. Monash & Woessner (126) subsequently reported that heat-inactivated enzyme preparations could still produce itching, and that cowhage which had deteriorated after long exposure to moisture and no longer caused itching still showed proteolytic activity. Arthur & Shelley (123) have attempted to explain these findings, but the available information is vague, and much more detailed biochemical and pharmacological study of specific substances is necessary. The possibility that smaller molecules may be concerned in the production of pruritus also requires further investigation; bradykinin, for instance, appears to be pruritogenic [Cormia & Dougherty (127)].

Local applications used clinically to relieve itching fail to alleviate itching induced experimentally by histamine or cowhage [Melton & Shelley (128); Macris *et al.* (129)]. Such applications will therefore have to be assessed by well-controlled observations on pathological pruritus.

Moulton *et al.* (130) have reported an intriguing observation on xanthosine, which regularly produces pain on intradermal injection. About half their subjects reacted with a flare, whereas the others did not. There may be a genetic basis for this difference. The relationship of the flare to histamine release and to wealing remains obscure.

Antimicrobial Substances and the Skin

Disinfection of the skin.—A recent review, in which Price (131) has authoritatively discussed the choice of a local antiseptic for use on the skin, has in the last year or so been supplemented by a number of papers from Britain. Lowbury and his colleagues have investigated methods of disinfecting the hands of surgeons and nurses (132) and the skin of operation sites (133); their results suggest several improvements on current preoperative routines. The prevention of staphylococcal cross-infection (134, 135) and of self-reinfection in chronic furunculosis (136) has been studied by Gillespie and his collaborators. Much of this work brings out the great usefulness of hexachlorophene.

Toxicity to skin and antibacterial effect.—Lawrence (137) has compared

the toxicity of various antibiotics to the skin in tissue culture by measuring its oxygen consumption and by examining it histologically. He suggests that a concentration found toxic *in vitro* may well impede healing if used *in vivo*. Collier & Grimshaw (138) have studied the local action of antibacterial substances in intact guinea pig skin by a different method. Each drug was injected intradermally, either on its own, or into the site of injection of an inoculum of *Corynebacterium ovis*, which gives rise to circumscribed circular lesions in the skin. When the drug was injected alone, the relation of lesion diameter to dose indicated the toxicity of the drug; when the organisms were also present, the results for nontoxic doses were a measure of the antibacterial effect of the drug. As the authors point out, the results are difficult to relate to clinical practice, but further development of the method using organisms frequently met clinically may help to narrow the gap.

Griseofulvin.—Griseofulvin is the first drug to be effective by mouth in the treatment of fungus infections of the skin. Its discovery represents a major advance which has been celebrated by two international symposia, in Miami in October, 1959 (139) and in London in May, 1960 (140). An editorial in the *Lancet* (141) provides a good review of the present position.

Clinical Pharmacology

Dermatological treatment, particularly external treatment, is still dominated by tradition. Most of the local applications in use are prescribed because they were recommended by some authority in the past, not because objective evidence justifies their composition. Frazier & Blank (142) recognized this and compiled an admirably brief formulary for external therapy of the skin. They included only preparations logically based on current scientific knowledge or, when this was inadequate, on a critical appraisal of the evidence. However, most of the preparations in Frazier & Blank's formulary have not yet been subjected to controlled experimental testing in patients, and firm conclusions can only be based on such tests. Although lip service has often been paid to this experimental approach, it appears that only Siemens (143 to 146) has made extensive use of a satisfactory method. His "one side treatment" method can only be used on patients in hospital and is time consuming, but these are small disadvantages because the results are of great help in the treatment of the individual patient as well as of general importance. The method depends on repeated treatment comparisons within each patient; comparisons between skin patients (i.e., groups treated differently) require far greater numbers and can give misleading results. Siemens' papers should be compulsory reading for all dermatologists.

Somewhat more is known about the effects on the skin of drugs given systemically. The classical studies of Bain and his collaborators (78, 81,

147, 148) on antihistaminics are still the most important if not the only reliable source of information on the clinical pharmacology of these drugs, particularly their relative potency and duration of action. These workers have investigated at least eight (antazoline, chlorcyclizine, chlorpheniramine, mepyramine, promethazine, thonzylamine, triprolidine, and "compound 405") of the 40 or more antihistaminic drugs on the market. Adequate data are not available on the human pharmacology of the other drugs, but this has discouraged few physicians from using them. Although good methods of evaluating drugs given systemically have been used in many branches of medicine, their use in dermatology has so far been exceptional. One such exception has been the clinical trial of griseofulvin in the treatment of chronic ringworm of the skin and nails [Russell *et al.* (149)]. To mention only two examples, the use of chloroquine in discoid lupus erythematosus and of dapsone in dermatitis herpetiformis needs to be studied by similarly rigorous methods; current practice is insecurely founded on poorly controlled observations.

Other Topics

The effect of detergents on the skin has been compendiously reviewed in the monograph by Stüpel & Szakall (150). A recent article by Bettley (151) has dealt with the effects of soap. Some effects of carcinogenic substances on the skin have been discussed by Walpole and by Berenblum in a Ciba Foundation Symposium (152).

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THE PHARMACOLOGY AND TOXICOLOGY OF THE BONE SEEKERS[1]

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INTRODUCTION

Bone as a tissue has been until recently largely neglected by pharmacologists. A major objective of this review is to call attention to the importance of the skeleton in any pharmacological or toxicological consideration. The extreme paucity of information, first as to what drugs go to bone and second as to how bone influences their pharmacological responses, does not reduce the potential importance of this organ system, but instead reflects only the current, inadequate state of our research methodology. A noncolored, nonfluorescent, or nonradioactive bone-seeking substance may go unnoticed or be lost in a distribution-excretion study as "per cent unaccounted for." As a matter of fact, it has only been since isotopes became generally available that great strides in our knowledge of bone metabolism have been made. If nothing else, it is hoped this review will stimulate pharmacologists to pay greater attention to the skeleton.

In this first annual review of the biological effects of the bone seekers, there is a great temptation to present an all-inclusive picture of the many interesting recent studies of the biochemistry of bone, its morphology, normal and abnormal physiology, and its unique importance in radiation biology. Even if such a review were possible within the brief confines of this chapter, however, an exposition of a few modern concepts to serve as an orienting framework of ideas should prove to be a more useful contribution. Such a framework will form a basis against which new information, now accumulating at an astounding rate, may be evaluated. For this reason an encyclopedic or annotated bibliography of scientific papers on bone seekers will not be presented; specific experimental data have been omitted. Also excluded are reports of substances or drugs that act primarily on the skeleton (vitamins and hormones) as well as discussions of the abnormal physiology of normal bone constituents, e.g., calcium, sodium, and magnesium.

Bone seekers as a group comprise a heterogeneous list; by broad definition this includes any substance localizing in the skeleton. Thus several col-

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loidal dyes of large molecular size (lithium carmine and trypan blue) have been shown to stain the organic material of new bone and to persist after decalcification procedures. Alizarin dyes and related anthraquinones also stain new bone by forming calcium lakes. The early studies on bone growth with madder were based on the red color of skeleton laid down during feeding of the dye.

Tetracyclines complex calcium in solution and have been shown to localize in bone by ultraviolet-fluorescence techniques. Although chemotoxicity to bone has not been observed at therapeutic levels, tetracyclines in large doses have been shown experimentally to inhibit embryonic bone development of chicks. Presumably many other substances not now recognized as bone seekers will ultimately be so classed when information on their distribution characteristics becomes available.

It is apparent that an inclusive discussion of the toxicology and pharmacology of bone seekers would be almost impossibly complicated. In this review, emphasis has been placed on two toxicological aspects: deleterious effect in the skeleton and systemic deleterious effects.

DELETERIOUS SKELETAL EFFECTS

A major impetus behind the research interest in the physiology and toxicology of the skeleton was the early recognition that many of the radioactive elements produced by nuclear fission deposited in this tissue. The deleterious effects of extremely minute concentrations of these elements were produced by virtue of their radioactive emanations. Thus, the great toxicological problem involving the skeleton per se is the assessment of the potential hazard to man of long-term low-level exposure to these nuclides.

Basic Physiological Concepts

Skeletal mechanisms.—The dynamic nature of the skeleton is well illustrated by its chemical reactivity. Since a significant portion of total bone is always in rapid equilibrium with blood and extracellular fluid, the importance of bone in any consideration of total body metabolism is understandable. As emphasized by Neuman & Neuman (1, 2) bone is, however, a highly specialized tissue and unique in many respects.

Total skeletal metabolism can be divided into three physiologically distinct compartments: (*a*) area of new bone formation, (*b*) area of bone resorption, and (*c*) stable bone. Children, depending on their age, have bone formation rates in excess of resorption which leads to net skeletal growth. Obviously, areas of bone formation and resorption must be in intimate contact with blood and extracellular fluid, and therefore these areas may be termed the "available skeleton." Stable bone, by definition at least, is not in contact with body fluids and this is the "unavailable skeleton." Incorporation of bone-seeking elements into the available skeleton accounts for the major fraction of skeletally deposited material.

At the microscopic level, turnover in adult compact bone proceeds pri-

marily through the process of haversian remodelling, i.e., the development of new osteones to replace those lost from resorption cavities through osteoclasis. Initially, these new osteones incorporate mineral at extremely rapid rates, whereas the completeness of mineralization of individual osteones varies with their age. In growing individuals, endochondral bone formation primarily at epiphyseal sites produces a relatively large volume of "available" trabecular bone. Diaphyseal membranous bone formation constitutes another component of the available skeleton. Mineral, including foreign bone-seeking elements, deposited at these sites may be derived in part from that removed from other sites by resorption.

About one-third of the fat-free dry weight of bone is composed of organic material. Only a small fraction is cells; most of the organic weight is the bone matrix which is impregnated with mineral. The major constituent (95 per cent) of matrix is collagen, similar in chemical composition to that from other connective tissue sources in the body, exhibiting a characteristic major cross-banding at 640 Å intervals. Based on x-ray diffraction and biochemical studies (3, 4), the role of collagen in the induction of biological calcification (nucleation) is becoming increasingly apparent. The remaining fraction of the matrix is the amorphous ground substance, consisting mostly of polymers of glucuronic acid and hexosamines such as chondroitin sulfate and hyaluronic acid. This component of bone has been least characterized and its importance has not been defined.

The major constituent of bone is mineral in the form of hydroxylapatite crystals, highly hydrated and with an extremely large total surface area resulting from their minute size. Thus, surface chemistry assumes paramount importance in bone physiology. The term hydroxylapatite refers to a particular type of crystalline structure, rather than to a specific chemical compound. Although usually represented by the formula $Ca_{10}(PO_4)_6(OH)_2$, the actual composition of bone mineral *in vivo* reflects the chemical composition of the immediate environment in which it was formed or with which it subsequently comes in contact. Depending upon their charge and ionic size, a number of ions may be substituted in certain positions of the hydroxylapatite lattice for Ca, PO_4, or OH, so that normal bone mineral contains varying amounts of Na, Mg, CO_3, and other ions in trace quantities.

Ion-exchange substitution constitutes the major mechanism for skeletal fixation of any bone-seeking element. Upon transport by the blood to the immediate environment of existing bone crystals, a rapid diffusion occurs into the "hydration shells" surrounding the individual crystals. From these hydration jackets, ionic exchange reactions may take place with Ca, PO_4, or OH ions on the crystal surface. For example, calcium exchanges on a 1:1 molar basis with Ca^{45} or Sr^{+2} and on a 2:1 basis for Ra^{+2} or UO_2^{+2}. The rate-limiting factor for this exchange in the case of the alkaline earths (Ca^{45}, Sr, Ra) seems to be the rate of "thermal escape" of calcium from the crystal surface, since these three ions of distinctly different sizes all enter the surface at comparable rates.

Following incorporation into crystal surfaces, certain ions are capable of incorporation into the crystal interior. The process of "recrystallization" is a relatively slow one, and only certain ions of proper ionic dimensions (Sr^{+2}, Ra^{+2}, F^{-}, and probably Pb^{+2}) can participate; others (Na^{+}, CO_3^{-2}, citrate^{-3}, Mg^{+2}, and UO_2^{+2}) apparently cannot.

Depending upon the particular substance, skeletally deposited materials soon become fixed by one or more of three processes: (*a*) recrystallization which essentially removes the isotope from surface exchangable sites; (*b*) apposition of new mineral so as to "bury" the ion in the crystal interior; and (*c*) a process termed "diffusion locking." As individual bone crystals grow by accretion of new mineral, these crystals with their highly oriented "hydration shells" attain such close proximity that the diffusion mobility of charged ions within or into the hydration layer is limited. Such ions are effectively excluded from further exchange reactions even though they may reside at surface positions.

Total exclusion of certain bone-seeking isotopes from "stable bone," however, does not occur. Recent histological evidence has shown that in addition to the exchange phenomena described above which require minutes or hours for completion, a process of "slow exchange" takes place (5). This usually requires one to two weeks to become apparent on autoradiographs as a remarkably uniform and diffuse low-intensity distribution throughout the entire bone sample, clearly differentiated from high-intensity "hot spots" (or new osteons) and other areas of new bone formation (i.e., the epiphyses). The exact mechanisms involved in this process are conjectural.

Neuman and co-workers (6) have recently proposed that a local lowering of pH is a mechanism for mineral mobilization. This is a physiological process directly related to parathyroid gland activity, which in turn is regulated through serum ionic calcium levels. Attempts to remove elements deposited in stable bone can only be truly successful through normal resorptive mechanisms. Only if the element were located exclusively on crystal surfaces in the available skeleton could one expect to remove it effectively with a chelator or exchange agent, thus explaining why such agents are efficacious only when administered before, during, or immediately after the exposure to the bone seeker. Since complete decalcification would be necessary to remove substances from stable bone (obviously unfeasible), the experimentally observed poor success of "skeletal detoxifying agents" (7, 8, 9) in late mobilization of skeletally deposited isotopes is understandable.

Blood transport.—The chemical nature of an element is of paramount importance in determining its physiological handling by the body. Obviously the entrant step is greatly dependent upon solubility as well as other factors. Once absorbed, the blood-borne element is transported to sites of deposition or carried to excretory organs for elimination from the body. Hydrolysis, colloid formation, formation of complexes with normally occurring ions or by binding to proteins may affect the behavior of the element. Although

there are great differences among the various groups of elements, striking similarities exist for elements of a given group. For example, the alkaline earths, calcium (10), strontium (11), and radium (12), are transported in plasma mostly in diffusible (mainly ionic) form. About 30 to 35 per cent of the total serum concentration of these alkaline earths is held as a nondiffusible, protein-bound moiety. Yttrium (13) and plutonium (14), on the other hand, occur in negligible concentration in the diffusible fraction; essentially all that present in blood is in nondiffusible form.

A dynamic equilibrium should exist between the free and protein-bound fractions so that any process which removes the diffusible components effects an instantaneous redistribution. However such an equilibrium probably does not apply in the case of nonfilterable complex colloids, and the importance of these colloids in blood transport and distribution is not well understood.

Excretion.—That portion of any element existing in diffusible form in plasma would be expected to filter through the glomerular membrane. Its appearance in bladder urine, however, would depend upon the relative contributions of reabsorptive and secretory mechanisms acting within the renal tubules. In general, these mechanisms, primarily reabsorptive, operate to conserve physiological ions (e.g., calcium) more efficiently than nonphysiological ions. Such mechanisms lead to "discrimination" against the foreign ion, a fortunate state of affairs especially in the case of Sr^{90}. The kidney discrimination against strontium can be explained in part by the lesser protein binding (leading to more efficient glomerular filtration) and in part by the more efficient tubular reabsorption of calcium. Unfortunately, attempts to improve on the natural renal discrimination against strontium have met with failure. Any process tested which interfered with renal reabsorptive mechanisms affected the physiological ion, calcium, more than the unphysiological ion, strontium (15).

Basic Radiological Concepts

Radiation injury.—Essentially all ionizing radiation, if absorbed, may produce reversible and nonreversible alterations of intracellular molecular structure (16). Even the nonreversible alterations generally do not result in immediately observable damage; rather the effects, in some unknown way, become manifest after a variable latent period or may be such as to produce no recognizable deleterious effects at any time.

Exposure to relatively large doses of externally applied whole-body irradiation (e.g., 300 to 500 r of x-ray or γ-radiation) produces certain characteristic symptoms and signs (17, 18, 19). These may be conveniently divided into: (*a*) acute effects, appearing within an hour after exposure and lasting for 24 to 48 hours, consisting of severe headache, nausea, vomiting, anorexia, irritability, and malaise; (*b*) early effects frequently following a few days of well-being and reaching a maximum in two to three weeks, consisting of bone-marrow depression, erythema, epilation, mucous membrane

ulceration, fever, and fatigue; and (*c*) late effects frequently requiring years to become manifest as cancer, cataracts, impaired spermatogenesis, genetic mutations, and early senility.

Radiation damage from a bone-seeking, γ-emitting radioisotope differs in no important respect from externally applied radiation (18). Gonads and other soft tissues as well as the bones would absorb the radiation dose. Qualitatively, however, radiation from an internally deposited α- or β-emitting isotope differs in many respects from the more penetrating γ-radiation (20). Because of the relatively short effective range of their emanations, local bone effects from α- or β-emitting bone seekers would far outshadow general somatic and genetic effects. Similarly, bone effects from the α-emitting isotope would be even more localized than those from the β emitter, and the latter would be expected to affect bone marrow to a relatively greater extent.

Another important consideration is the marked nonuniformity (21, 22) of skeletal uptake of all bone-seeking agents, discussed previously. Since radiation effects are proportional to absorbed dose, this nonuniform distribution assumes great importance in evaluating potential radiation hazards and greatly complicates estimations of the precise radiation dose delivered to the skeleton by α and β emitters (23, 24, 25).

A given dose of radiation insufficient to produce acute or early effects may still give rise to a variety of manifestations. The incidence of these late effects decreases as the dose decreases; the lower limit is the natural background radiation from our environment (cosmic rays, soil, etc.). Of great present concern (26, 27, 28) is the assessment of dangers from low levels of radiation which produce an extremely low incidence of observable effects. Of necessity, the preponderant data have been obtained on radiation levels above the levels of interest, i.e., maximum permissible exposure allowed for occupational exposure and radiation dose from Sr^{90} fallout. To extrapolate to the lower dose levels, it should be known whether the dose-response curve is linear or nonlinear and whether there is a threshold or no threshold for the radiation effect (29). Inspection of available experimental data reveals a scatter of points which allow no immediate and simple answer. According to the specific data consulted, lines can be drawn to prove any of the four possibilities, three of which must be wrong for any individual radiation response considered. The threshold concept itself is subject to significantly varying interpretation so that the threshold "to what effect" must be rigorously defined (30).

In assessing the risks of low-level radiation (i.e., for fallout), reasonable conclusions can be reached only if extremely large populations are studied; but it is doubtful whether with the gross criteria now used we will ever be able to demonstrate statistically valid effects on the human population (31). For example, two of the recognized carcinogenic effects associated with large doses of bone-seeking isotopes are leukemia and bone tumors. Granted that the general population has not been exposed to radiation levels

much above prenuclear age background, we turn to workers in the atomic industry. These in the United States have been estimated at 0.25 per cent of the population, which, with a normal incidence of these tumors would mean only nine individuals per year with bone tumors and 30 with leukemia—a clearly insufficient base on which to pass statistical judgment. The formidable task of directly assessing radiation risk to man is apparent. More sensitive indicators of cellular or functional damage are clearly needed (24, 32).

Radium as a standard.—The paramount importance of radium in assessing radiological hazards of bone-seeking isotopes follows from the fact that a large body of direct clinical observations at a wide range of dose levels have conclusively established that this radionuclide has deleterious effects on the human skeleton (21, 32, 33). The metabolism and skeletal uptake (34) of radium resembles the other alkaline earths, calcium and strontium. Since no such comparable data are available for other radioactive bone seekers, potential hazards must be assessed against the background of the radium experience.

Body burdens above 1.0 μg. radium^{226} are clearly hazardous. With very high Ra doses, effects may become manifest early, as was seen in some dial painters four to six years after exposure, typified by severe anemias and by necrosis of the bones of the jaw. These effects may be clearly differentiated from those which are produced after a longer latent period. In a high percentage of cases, there were roentgenographically detectable bone changes, microscopic and macroscopic necrosis, and bone-tumor development 10 to 20 years after initial exposure. Yet it should be pointed out that some individuals having a present body burden considerably above 1 μg. Ra^{226} (up to 14 μg.) show no detectable evidence of deleterious effects even after several decades of exposure.

In the range 0.5 to 1.0 μg. radium, statistically fewer individuals exhibit toxic effects. The lowest level at which minor roentgenographic changes have been described has been in a patient who after 20 years had a body burden of 0.4 μg. Major skeletal damage in other patients has occurred as low as 0.7 μg., and one case of neoplasia has been found at 0.8 μg. No detectable changes have been recorded below these levels (32).

The radium^{226} maximum body burden was set by an advisory committee to the U. S. National Bureau of Standards at 0.1 μg (0.1 μc.) in 1941 and has remained unchanged although there is not complete agreement on this point. This allowable body burden of Ra^{226} for occupational exposure is a thousand times the usual content of about 0.0001 μg. Ra in the adult (35) derived from natural sources. An important consideration, tending to suggest that this value is a conservative one, is that all available data are based on estimations of the body burden several decades after the presumed initial exposure. The 0.1 μg. "radium standard" is thus a terminal value and extrapolations utilizing one of several formulae are necessary to estimate the much greater original dose. Furthermore, the original dose in many in-

stances, especially in the dial painters, was not pure Ra^{226} but contained significant quantities of Ra^{228} (mesothorium) with the short half-life of 6.7 years which therefore could not be detected at the time most radium patients were studied. Contamination with mesothorium would greatly increase the predicted toxicity, for not only would the initial dose rate have been much higher, but the relative toxicity of mesothorium has been reported to be much greater than radium. Finally, the 0.1 μg. of Ra^{226} refers to a maximum value reached after 50 years of chronic occupational exposure rather than an initial permissible dose. The total radiation dose delivered through chronic exposure over the 50-year period should be much less. Also a more uniform distribution throughout the entire skeleton would be expected since areas of high concentration in bone as a result of acute administration would be avoided. Thus the 0.1 μg. maximum body burden for occupational exposure may contain a built-in safety factor of two- to fivefold.

On the other hand, it has been pointed out that although there are many data for very low and very high Ra body burdens, few cases with body burdens at or near the recommended maximum body burden of 0.1 μg. have been studied, and the period of observation has not extended in any instance through an entire normal human lifespan. Techniques for detecting early or minor histopathological changes are not available so that the ultimate safety of the Ra^{226} standard has not been unequivocally established.

In any event, the basic method used for estimation of the maximum permissible body burdens for bone seekers other than radium is to calculate the quantity of such an isotope which would deliver to bone a dose in rems equivalent to that delivered by 0.1 μg. Ra^{226}. Even though the organ burden of Ra is assumed, a precise calculation of the exact dose delivered to the skeleton still remains extremely difficult (36). The dose delivered at each step in the decay sequence of radium must be individually calculated and is complicated by the escape of radon (and consequently its daughter elements), which results in considerable loss of energy otherwise absorbed by bone. Such calculations have given a dose rate of 0.06 rads/week (corresponding to 0.56 rems/week) for 0.1 μg. Ra^{226} which is the figure accepted by the International Committee for Radiation Protection (ICRP) (37).

Essentially 96 per cent of the total dose delivered by radioactive decay of Ra^{226} is contributed by α particles (38). To apply such data to β- and γ-emitting bone seekers requires the further application of a correction factor taking into account the well-established observation that equal doses of different kinds of radiation may produce varying biological effects. This has been termed the relative biological effectiveness. Precise estimation of this factor by a physiological end point is not simple but depends on the exact physical and physiological conditions employed. Consequently, the relative biological effectiveness for α particles is based on a purely physical measurement (ion densities along particle track), and a value of 10 has been recommended by the ICRP (37, 38).

After correcting the delivered dose in rads for relative biological effec-

tiveness to obtain a presumably equivalent dose in rems, equal doses of various bone-seeking isotopes still exhibit significantly different toxicities. Reasons given for this discrepancy include (*a*) less uniform distribution of the istotope in question as compared with radium and (*b*) greater radio sensitivity of its different sites of deposition. To offset this difference, a "relative damage factor" termed "n" was introduced to correct for the presumed increased hazard for a non-γ-emitting bone seeker (*37*, 38). The value of n is one for Ra and γ emitters and is five for all other isotopes; thus, equal rem doses of Sr^{90} or Pu^{239} appear to be five times as carcinogenic as Ra^{226}, and the n factor takes into account this observed difference. The final calculated value is then called the relative biological effectiveness dose. It should be pointed out that the relative damage factor is being applied by the ICRP in calculations of maximum body burden for all bone seekers by the "radium dose method," but at dose levels far below the experimental levels on which the factor is based.

An alternative method is employed for calculating maximum body burden of bone seekers emitting only γ-radiation where bone itself might not be the critical organ. It is also used for all nonbone-seeking radionuclides. The critique is to limit the weekly dose rate to various organs of the whole body to the limits set by the ICRP for external irradiation [i.e., 0.1 rem/week to gonads and whole body, 0.6 rem/week for skin and thyroid, and 0.3 rem/week to other soft tissues (*37*)]. Thus the maximum body burden for Sr^{85} which emits only γ-radiation would be based on a 0.3 rem/week since adjacent soft tissues would be irradiated in addition to bone.

All the above calculations assume a uniform distribution of the radioisotope throughout the skeleton. As pointed out under basic physiological concepts, such an assumption cannot be valid because of the markedly different reactivity of the various skeletal compartments. Local dose rates delivered to bone, marrow, or surrounding tissues would depend on the degree of this inhomogeneity of isotope deposition and also on the type and energy of radiation emitted.

Upon consideration of the $Sr^{90} - Y^{90}$ decay process and the geometry of hot spot vs. diffuse labelling, it has been calculated (*23*, *25*) that, although 2 μc. of Sr^{90} would give a dose rate of 11.6 millirad/day if uniform labelling were assumed, the dose rates delivered to marrow and bone range from below 0.5 to perhaps 100 millirad/day, dependent on whether the isotope deposits from chronic (labelling of all mineral) or acute administration (highest labelling in available skeleton). Thus, a certain fraction of bone and marrow would receive doses far above the average dose calculated for uniform deposition.

Translocation of radioactive elements.—The translocation of skeletally deposited isotopes within bone as remodelling occurs has been mentioned. In addition to this transfer of a given isotope from one microscopic site to another site in the same tissue is the possibility of translocation from one site to another as a radioactive element transmutes into another element by

decay. If the decay product is nonradioactive, no problem exists, for the mass of product would be negligible. If, however, the parent element forms a radioactive daughter, then this daughter must be taken into account in all calculations of dose and hazard estimation.

Short-lived daughters constitute a special case. For example Sr^{90} decays with a half-life of 28 years to Y^{90} with a physical half-life of only 64 hours, which is short compared with the time it would require for the body to mobilize the Y^{90} and deposit it in the typical pattern of a rare earth. Release of energy for Y^{90} thus occurs primarily at the original site of Sr^{90} deposition. Similar situations exist for other short-lived daughters.

Radon, a noble gas, is mobilized from bone to expired air relatively rapidly compared to its physical half-life of 3.8 days. Such translocation effectively removes radon and its radioactive daughters from consideration in dose calculations of delivered energy from Ra^{226} decay (38), and measurement of expired radon can be utilized to estimate radium body burden.

The special case where a radioactive daughter may translocate within bone, or between various sites of deposition is illustrated by thorium. Isotopes of this element are members of each of the three natural radioactive decay series: uranium, thorium, and actinium. In each case thorium decays to an isotope of radium, hence the physicochemical and physiological behavior of these thorium daughters is similar to Ra^{226} instead of the parent thorium (39).

Uranium as an Example of a Bone Seeker

No better example of the multifaceted nature of the pharmacology and and toxicology of bone seekers can be presented than that derived from a consideration of uranium. With the advent of nuclear weapons, great impetus was given to a comprehensive physiological and radiological study of this essential fissionable nuclide (40).

Three isotopic forms of uranium (U^{234}, U^{235}, U^{238}, with a relative abundance of 0.006, 0.71 and 99.58 per cent, respectively) occur in nature as relatively insoluble ores. Being α emitters, all are naturally radioactive; but because of differences in half-life, on a weight basis the α activity of U^{234} is approximately 17,000 times, and U^{235} six times, greater than the abundant U^{238}. Similarly, the artificially produced nuclear fuel, U^{233}, has an α activity approximately 27,700 times that of an equal weight of U^{238} (41). Thus, the degree of radiotoxicity depends directly upon whether one is dealing with natural uranium, as in the mining industry, or with the "enriched" uranium utilized for reactors or weapons.

The chemotoxicity of uranium has been well documented (40, 42, 43). The kidney is principal site of uranium injury after it once gains entrance to the circulation. Although several valence forms of uranium exist, the hexavalent, uranyl ion (UO_2^{+2}), is of primary physiological importance in the aqueous environment of the body. This ion complexes readily with various normal organic and inorganic anions which in turn may be diffusible (e.g.,

principally bicarbonate complex) or nondiffusible (plasma protein complex). As with calcium (10), approximately one-third of the plasma uranium is protein-bound and in dynamic equilibrium with the diffusible forms (42).

Under physiological conditions, normal uranium in complexed form is nontoxic. However, if free uranyl ions are present, combination with surface-located polyphosphate groupings may occur and interfere markedly with normal cellular functions (44).

After intravenous administration of uranium, approximately 60 to 70 per cent appears in the urine during the first 24 hours (45). Thus if an acid urine is being elaborated with concomitant reabsorption of bicarbonate, the filtered uranyl bicarbonate complex dissociates upon reaching the proximal convoluted tubule, allowing free uranyl ion to combine with tubular epithelial cells. Severe renal tubular damage may result. Clearly, then, this bone seeker has a pronounced tissue toxicity quite apart from its potential toxicity to the skeleton. In fact, the calculation of the maximum permissible dose of natural uranium, as well as U^{238}, is based on the kidney chemotoxicity. With more active α-emitting uranium isotopes, U^{233}, U^{234}, U^{235}, the bone becomes the important organ of concern from the radiotoxic standpoint (37). Uranium^{238} can never become a radiological hazard in man because the doses necessary to deposit enough uranium in bone to equal in radiation that delivered by 0.1 μc. of radium would be far in excess of the uranium doses proving lethal by kidney destruction.

These considerations apply to uranium once it has gained access to the circulation. As with all bone seekers, exposure may be to soluble forms, insoluble forms, or both, and three possible portals of entry exist; viz, the gastrointestinal tract, the lung, or the skin. When in a soluble form, rapid absorption may occur through any of these pathways. However, uranium and the majority of radiotoxic bone seekers, with the notable exception of strontium, are poorly absorbed from the gut or unbroken skin in any form (40, 42, 46). If gastrointestinal absorption does not occur, the element whether soluble or insoluble is soon excreted, whereas pulmonary deposits of a poorly absorbed material (insoluble uranium dust, for example) are only slowly mobilized. In this case then, the lung assumes critical importance in assessing the radiotoxicity from the bone seeker (47).

Fallout and Strontium^{90}

Fallout.—The current intense interest in the metabolism of strontium in man is a direct consequence of the development of nuclear weapons. About 200 radioactive isotopes of more than 30 elements are disseminated in a nuclear explosion (48). In addition to particles of the original fissionable material (uranium or plutonium), these consist of radioactive fission fragments of elements occurring primarily in the middle portion of the periodic table. Other isotopes may be created by the strong neutron flux associated with the chain reactions, the most important being the conversion of atmospheric nitrogen to carbon^{14}.

The radioactive elements formed during the explosion fuse with varying amounts of wholly or partially vaporized debris at the test site resulting in particles of widely different sizes (49). Depending on the force and the location of the explosion, various quantities of the smaller particles may be injected beyond the troposphere (i.e., the air layer extending 35,000 to 55,000 feet above the ground in which the earth's weather conditions are determined) into the stratosphere. Thus with an air burst of a weapon one megaton (TNT equivalent) or greater, it is estimated that 99 per cent of the radioactive debris enters the stratosphere and 1 per cent remains in the troposphere. A water surface or coral surface burst injects only 30 per cent and 20 per cent, respectively, into the stratosphere (50).

Fallout from the explosion may then be immediate (local), intermediate, or delayed (19, 51). Local fallout consists primarily of the large particles reaching the ground within the first few hours. Wind conditions at the time may, however, distribute the debris over a wide area as in the case of the 1954 Marshall Island Tests.

The smaller particles not reaching the stratosphere are rapidly carried away from the test site by air currents. They may circle the earth several times before being carried to the ground, primarily through rainfall, as intermediate fallout. This generally occurs within the first few weeks or months, and the distribution is within the same general latitude as the explosion site.

The small particles injected into the stratosphere constitute the delayed or "world-wide" fallout. These particles only slowly re-enter the troposphere from which they are subsequently carried to the ground through rainfall. The mean residence time of stratospheric debris is now estimated to be considerably less than the older 5 to 10 year estimates. Except in the event of all-out nuclear war where local and intermediate fallout assume great importance, it is the delayed fallout which has created world-wide concern (18, 26, 27).

Measurements one hour after a one megaton detonation indicate that approximately 300,000 megacuries of radioactivity (49) are produced in addition to variable quantities of radioactivity resulting from the associated neutron bombardment. Of the hundreds of different radioisotopes which enter the stratosphere for delayed fallout, most have relatively short radioactive half-lives (minutes, hours, days) or consist of relatively insoluble oxides (i.e., $Pu^{239}O_2$, $Ce^{144}O_2$, $Ru^{106}O_2$, and $Zr^{95}O_2$) and therefore do not constitute an ingestion hazard (52). To constitute a potential hazard, a stratospherically deposited isotope must combine a long radioactive half-life (years) with (*a*) a high fission yield, (*b*) a chemical form premitting its ready incorporation into the food chain leading to man, and (*c*) known toxic effects at certain dosages. Of the isotopes produced through fission reactions, cesium^{137}, carbon^{14}, and strontium^{90} have been, until very recently, the isotopes of primary concern.

Cesium^{137} with a half-life of approximately 30 years, has fission yield of

about 6 per cent from the slow neutron fission of uranium^{235} and, being very similar to potassium, is virtually completely absorbed from the gastrointestinal tract. This isotope and its decay daughter, barium^{137}, emit relatively strong β- and γ-rays and constitute a potential genetic hazard by virtue of its distribution throughout the entire body, including the gonads. Recently, active discussions have taken place concerning the potential genetic and somatic hazards of carbon^{14} to the world's population (53, 54). This isotope is formed through the interaction of fission-produced neutrons on atmospheric nitrogen, i.e., $N^{14} + n \rightarrow C^{14} + p$, and appears as $C^{14}O_2$. Its extremely long half-life (5570 years) and ubiquitous distribution throughout the biosphere, including the genetic material of plants and animals, are the important factors in these considerations. From weapons tested thus far it has been estimated that approximately 0.56 tons of C^{14} have been added to the 44.3 tons existing before testing. The latter appears normally in our biosphere from the constant bombardment of nitrogen by cosmic rays.

Strontium^{90}.—Of primary interest to this discussion, however, is the strontium^{90} contribution to long-term fallout. Strontium^{90} is chemically similar to calcium and satisfies all the hazard criteria enumerated above. In addition, the isotope is concentrated only within bone. Unique characteristics have placed strontium^{90} into a position where it is feared as a serious fallout hazard.

It has been estimated that in the fission of uranium or plutonium approximately 3 to 5 per cent of the fissions result in the formation of a krypton^{90} atom. Its subsequent decay then occurs through the following sequence: krypton^{90}—33 sec.→rubidium^{90}—27 min.→strontium^{90}—29 yrs.→yttrium^{90}—64 hrs. → zirconium^{90} (stable) (55). The importance of this reaction scheme lies in the fact that the actual production of strontium^{90} occurs relatively late, and its precursor, a noble gas, is distributed widely immediately after the explosion. Condensation of radioactive nuclides to test-site debris particles is thought to occur within the first minute (56). Thus local trapping of strontium to prevent stratospheric contamination is extremely difficult. (This is also true of cesium^{137} which is formed through the decay of another noble gas, xenon^{137}.)

Of critical importance in assessing long-term fallout hazard from strontium^{90} are (*a*) the percentage of the total amount of strontium^{90} produced that has already been injected into the stratosphere and the expected contribution from future tests, (*b*) the rate at which this stratospheric inventory reaches the earth's surface, (*c*) the degree of uniformity or nonuniformity of fallout deposition on the ground, (*d*) the extent to which fallout strontium^{90} reaches the internal organs of man, and (*e*) the concentration of strontium^{90} that is toxic to the human organism. All but the last factor enter into the calculations of predicted future levels of strontium^{90} in human beings. Regardless of the accuracy of such predictions, of equal or greater importance is the last-mentioned consideration, i.e., the assessment of the potential hazard of low-level quantities of strontium^{90} in bone.

During the past few years, lively debates have centered around each of these points with national and international repercussions (26, 27). Four years ago, relatively little data of quantitative nature were available. Assumptions, extrapolations, or estimations were necessary for each of these considerations and, depending upon how optimistic or conservative one wished to be, a safe or serious hazard prediction could be drawn. Since that time more accurate information has become available for strontium^{90} in the first five categories so that the initial sharp controversies have disappeared (57).

Original estimates of the amount of strontium^{90} injected into the stratosphere were based upon fairly good approximations of the number of megacuries produced per megaton fission yield minus the amount trapped at the site. For bombs exploded by countries other than the United States, the latter figure could only be approximated. The actual stratospheric inventory during a given year was obtained by subtracting the amount of strontium^{90} (estimated by soil analysis) already deposited on the ground. Such calculation gave a value of two to five megacuries in 1959 (19). No further megaton testing has occurred since the Russian series in October 1958. Direct high-altitude balloon and aircraft sampling has since shown that this was an over-estimate, the stratospheric inventory being approximately 0.6 megacuries in the fall of 1959 (58).

Another over-estimate was the length of the mean residence time of stratospheric debris. Whereas five to 10 years was originally given, it now appears that this may actually be less than one year and certainly less than three years. Recent studies have also confirmed Machta's original prediction of nonuniform distribution of global fallout, about two-thirds of the total appearing in the northern hemisphere in addition to uneven distribution within the hemisphere. Seasonal variations in fallout rate have also been noted (57, 58). Of present concern is the significance of geographical "hot spots," i.e., localization of relatively high environmental radioactivity within a particular country or state. These are thought to result from the combination of a high concentration of tropospheric nuclear debris, over a particular geographical area, coincident with heavy rainfall (59). The above-mentioned meterological considerations have led to a renewed interest in the biological hazards of short-lived fission products such as Sr^{89}, Ba^{140}, and I^{131} (60).

Of major concern now is the estimation of the risk of late effects of low-level strontium^{90} irradiation. Strontium is an alkaline earth appearing in the periodic tables just below calcium so that the chemical and physiological behavior of these two elements is similar, though not identical (61 to 64). In most biological processes there is a distinct preference for calcium assimilation and utilization. The extent to which this "discrimination" occurs varies throughout each of the steps in the fallout-plant-animal food chain leading to man (50). Studies designed to determine the magnitude of these factors assumed great importance in the original predictions of future levels

of strontium^{90} in human bone. Because specific information was not available, the over-all discrimination factor against strontium^{90} (soil to bone) accepted by various investigators varied from four to 20 (57). The weakest link in the chain appeared to be that from soil to plant. In any event, it was necessary to assume in all such calculations that the soil-plant factor remained constant and was related to the concentration of strontium in the soil (expressed in strontium units where one strontium unit equals one "sunshine" unit equals one μμc. Sr^{90} per gram calcium). This in turn depended upon total accumulated fallout. It is now clear that there is no constant soil-plant discrimination. This is because the major source of strontium^{90} in plants is through direct fallout contamination (i.e., absorption through leaves and plant base absorption) so that "dilution" with soil calcium does not occur (65). Thus, fallout rate, rather than accumulated total fallout is the significant consideration, and, as indicated above, this has now been shown to be quite variable. In addition, gastrointestinal uptake of strontium^{90} varies not only with the dietary intake of calcium, but with other materials in the food (almost 99 per cent of the strontium^{90} carried to the ground by rainfall is retained by soil and plants so that drinking water is not considered a source of contamination).

With the availability of the recent data cited above, it now appears that predictions of peak levels of strontium^{90} in ground, diet, and human bone made even within the past one to two years were too high and set too far in the future. The most recent calculations (66) indicate that maximum ground and diet levels from previous tests occurred in 1959 since 4.5 of the estimated 5.1 megacuries injected into the stratosphere had already reached the ground. Maximum strontium^{90} concentrations in human bones were predicted for 1960. For the latter, the values expected in individuals 1, 15, and 26 years old in western culture areas of the northern hemisphere are approximately 2.6, 1.2, and 0.4 μμc. Sr^{90} per gram calcium, respectively. Assuming no further testing, these authors calculated that in 1970 (presumably when equilibrium between biospheric strontium^{90} and the skeletal systems of the human population will be achieved) human bones should average between 0.5 to 1.0 μμc. Sr^{90} per gram calcium. Still to be determined is the degree of statistical variability throughout the world's population and the specific effects of low calcium diets (e.g., rice-diet countries) and "hot spot" areas. In 1959, Langham suggested a spread of five to 10 times the then available predicted mean values (50).

Ample evidence exists in the literature to show that strontium^{90} is carcinogenic in laboratory animals (67, 68). Rough dose-response relationships have been obtained and differences in tumor incidence observed between single intravenous administration and continuous ingestion. A clear dependence of biological effects on dose-rate exists (69). Biological variables such as species, strain, and age of the experimental animals have been found to significantly affect the results (70). While yielding valuable information on the pathological effects of large doses of radiation per se, the major diffi-

culty lies in the extrapolation of such data to low levels of radioactivity in man (67, 71). All animal studies have involved doses of strontium^{90} in the order of 0.1 to 1.0 μc. per gram body weight or intakes of 10 μc. strontium^{90} per gram calcium. As pointed out by Lamerton (72) the mean skeletal dose in those animals developing tumors would be in the order of thousands or tens of thousands of rads. No laboratory information is available on the risk of bone-tumor production from radiation doses less than several hundred rads.

An unresolved question is the extent to which the natural radiation background (cosmic rays, Ra^{226}, K^{40}, C^{14}, etc.) contributes to the normal incidence of tumors and genetic mutations. Opinions vary from "all" to "few." If this incidence were known with any degree of certainty, extrapolations upward for the added dose delivered from fallout would enable precise evaluation of the hazard, since the natural background radiation to man is known with relative accuracy (approximately 0.13 r per year or 7 to 10 r over a 70-year lifespan). The presently recommended maximum body burden of strontium^{90} for the general population set by the ICRP (37) (equivalent to 67 μμc. Sr^{90} per gram calcium) follows from accepting the risk of doubling the natural background dose.

One μμc. Sr^{90} per gram calcium homogeneously distributed delivers a radiation dose rate to bone of 0.003 rads per year (50). Thus, if predictions of Kulp and co-workers (66) prove correct and if no further megaton testing occurs, equilibrium values for strontium^{90} in bone will be far below the ICRP recommended maximum. While it may subsequently be shown that the original concern about the hazard of the long-lived strontium^{90} was unduly great, it did nevertheless stimulate intensive research. Within the space of a few years many important questions were resolved. On the other hand, new problems were brought to light. For example, the newer meterological data have focused renewed attention on the shorter-lived fission products previously not considered as systemic hazards (60).

Members of Inner Transition Series

A large number of bone-seeking elements are members of the two inner transition series of the periodic table. In these elements the outer valence electronic shells are similar, but the inner levels are incompletely filled (73), and one element differs from another only by electrons in one of the inner d or f levels. Thus, only slight differences in physicochemical behavior will become apparent, since the important valance shells are unchanged.

The first such series, with distinguishing electrons in the 4-f orbitals are called the "lanthanides" or rare earths, and the strict electronic definition is commonly broadened to include the very similar yttrium, lanthanum, and lutetium. Elements following radium with distinguishing 5-f orbitals have been classed (73) as members of a second inner transition series and termed "actinides." These include actinium, thorium, uranium, plutonium, americium, curium, and elements subsequent to No. 103.

Similarities in physicochemical properties between members of each group form a convenient basis on which to discuss the physiological behavior of these elements. Uranium has already been mentioned as a bone seeker ordinarily not capable of gaining access to the circulation except by accidental or intentional injection or by inhalation. Similar situations exist for the other actinides and the lanthanides. However, because of the extensive industrial use of some of these elements and their occurrence in fallout, considerable experimental attention has been directed to these materials.

The rare earths.—A multitude of radioactive isotopes of the rare earth group is produced in fission reactions (26). Their potential danger to man has been said to be small because of their extremely poor absorption (0.01 per cent) upon ingestion (74) and their relatively short half-lives. Total radioactivity remaining 20 days after slow neutron fission of one kg. of U^{235} amounts to about 10 megacuries. About half is present as rare earth isotopes, including lanthanum^{140}, praseodymium^{143}, yttrium^{91}, and neodymium^{147}, with half-lives of the order of several days or weeks. Essentially none of the radioactivity remaining two decades later would be as any of these isotopes (26). It must be pointed out, however, that several of the longer-lived rare earth isotopes could be predicted to contribute a significant fraction to the 10,000 curies still remaining at 20 years. Promethium^{147} with a 2.6-year half-life and samarium^{151} with a 98-year half-life would contribute 3.4 per cent and 2.6 per cent, respectively, compared with the 48 per cent attributable to $Sr^{90} — Y^{90}$. All the rare earths deposit in the skeleton, the heavier members of the series, e.g., thulium^{170}, depositing to a greater extent (75). The skeletal distribution pattern is distinctly different from that of the alkaline earths. Earlier interpretations of an organic matrix distribution were incorrect, for it is now known that yttrium *in vitro* does not bind bone matrix but selectively deposits on bone mineral or hydroxylapatite. The physicochemical solution behavior of yttrium has recently been utilized to explain its preferential deposition in highly calcified nongrowing resorption cavities rather than actively calcifying sites (6).

In neutral solutions, yttrium, unlike the soluble and largely ionic alkaline earths has a strong tendency to hydrolyze and form radiocolloids (13). Furthermore, protein binding of yttrium in serum renders it practically nonultrafilterable (0.5 per cent). Under conditions of either lower pH or increased citrate concentration, or both, yttrium is rendered much more diffusible, precisely the conditions postulated for resorbing bone sites mentioned under basic physiological concepts. Mineral exchange reactions then proceed in the usual way. By inference, other rare earths with similar physicochemical properties would also be expected to deposit at resorbing sites where these unique conditions of pH, complexing ion concentrations, or both, appear to exist.

Plutonium.—Plutonium^{239} is an α- and γ-emitting isotope with a physical half-life of 24,400 years. Since it is one of the primary fissionable fuel elements, plutonium^{239} is produced in large quantities from U^{238} for use in

nuclear reactors and weapons and thus constitutes a potential industrial hazard (76). Although absorbed only 0.003 per cent from the gastrointestinal tract (77), 1 to 10 per cent of an inhaled dose may be absorbed depending on the physicochemical properties and size of the particles. Absorption through skin and accidental punctures also may introduce plutonium into the body.

Absorbed plutonium is transported in the blood primarily as nondiffusible complexes (14) with serum globulins or as colloids, from whence it is rapidly fixed by skeleton and liver. If PuO_2^{+2} complexed with citrate or versenate is administered, skeletal deposition is much greater than liver (70 per cent vs. 15 per cent) whereas uncomplexed Pu^{+3} or Pu^{+4} initially deposits more or less equally between the two organs. Complex colloid formation at physiological pH accounts for the greater reticuloendothelial system fixation in the latter instance.

Microscopically, plutonium fixation by the skeleton is highly localized (78) in a nonuniform manner on endosteal and periosteal bone surfaces and in regions of trabecular bone. Unlike radium, this distribution does not become as diffuse with time.

Although the specific activity of Pu^{239} (0.064 μc./μg.) is lower than that of Ra^{226}, the maximum permissible body burden of 0.04 μc. (37) is lower than that for Ra^{226} because of higher α energy (Eα equals 5.15 Mev) and apparently greater radiotoxicity mentioned previously (n equals 5) resulting from the highly localized distribution.

Excretion from skeletal deposition sites is extremely slow with a half-time of about 200 years. In man and dog the urinary excretion is of greater magnitude than via the feces.

Thorium.—Until recently, the most widely used radioactive element in the medical field was the actinide, thorium. Thorium dioxide (Thorotrast) was administered intravenously as a radio-opaque x-ray contrast medium and, being an insoluble colloid, was fixed by the reticulo endothelial system organs: liver, spleen, and bone marrow. Very little translocation of radium daughters from thorotrast was observed (79).

Present interest in thorium revolves around its importance as a nuclear fuel. Although not fissionable itself, Th^{232}, which comprises essentially all of the naturally occurring element is a fertile material like U^{238} and is converted by slow neutrons in a "breeder" reactor to U^{233}, which is fissionable. If intravenously administered as the soluble quadrivalent citrate complex, thorium is bound by the skeletal system (80 per cent) in a very firm manner similar to plutonium (80). Radium daughter isotopes formed by decay, i.e., mesothorium I (Ra^{228}), are handled much like intravenously injected radium, with considerable urinary excretion and deposition in the available skeleton (39). The radioactive hazards from natural thorium include Th^{228}, the radioactive granddaughter of Th^{232}, with a comparatively short half-life of 1.9 years. Thus, the ICRP maximum permissible body burden for natural thorium is only 0.01μc. compared with 0.04 μc for Th^{232} (37).

DELETERIOUS SYSTEMIC EFFECTS

The remainder of this review has quite a different approach. Attention is limited to three bone seekers—fluoride, beryllium, and lead—which have important systemic effects: Fluoride is selected for its extraordinary public health importance; beryllium because its unmatched metallurgical properties are coupled with insidious and mystifyingly supertoxic properties; and lead to point up a major problem in the toxicology of the future. For each element the effects on the skeletal system are not important or not stressed.

Fluoride

In this country, fluoridated water is now consumed by communities whose total populations exceed 30,000,000. Despite the wide acceptance there still remain groups including a few medical and dental practitioners who oppose this practice for a variety of reasons. Because a great deal of information is available (some 4000 papers on the biological effects of fluorides have been published since 1935) and because the physician and dentist occupy strategic positions in this community program, a brief summary of the pharmacology and toxicology of the inorganic fluorides is presented.

Practically all foods contain traces of fluoride; the average individual probably ingests 0.5 to 1.5 mg. F/day of food-borne inorganic[3] fluoride (81). Relatively insoluble inorganic fluorides, such as, calcium fluoride or bone meal, are understandably less efficient fluoride sources than the completely absorbed soluble fluorides, such as sodium fluoride (82). Fluoride dissolved in a hard water is just as available as the same concentration of fluoride in a soft water. It is none the less true that calcium, like aluminum, and some other substances tend to slow down and reduce the absorption of fluoride into the body. Thus, fluoride in milk is slightly less available and more slowly absorbed than the same amount of fluoride in water (83). Industrial exposures to fluoride-containing dusts serve as sources of fluoride (84, 85).

Regardless of the combination in which inorganic fluoride enters the gastrointestinal tract or the lungs, only fluoride ion is absorbed into the circulation. Fluorides are rapidly distributed through the extracellular fluid and probably through all body water in a pattern nearly identical with that of chloride (86 to 90). In a previously unexposed individual, the distribution pattern is simple: about half the fluoride is promptly deposited in the skeleton, about half promptly excreted in the urine. No soft tissue stores fluoride. Salivary fluoride concentrations are low, approximately equal to those in blood. Milk contains only traces of fluoride. Fluoride crosses the placenta

[3] The biological effects of the organic fluorides (found in certain South African plants; synthetic products) are not considered in this chapter. Their actions are of considerable theoretical and practical interest. The reader is referred to *Toxic Aliphatic Fluorine Compounds* by Pattison (92); to *Phosphorous and Fluorine* by Saunders (93); and to the current clinical literature for information about compounds in clinical use, such as, fluorosteroids, and fluorinated agents used in advanced neoplastic disease.

and the fetal blood fluoride concentration is approximately equal to that of the maternal blood (91).

From the lengthy list of real or purported effects of fluorides that can be culled from the literature, a few are selected that have been studied in sufficient detail so that quantitative information is available (Table I). It is important to note that the time during which the fluoride effect occurs is almost as important as the amount of fluoride.

Vegetation damage in the vicinity of industrial plants from gaseous or dust exposures to fluorides has long been known. Gladiolus, for example, and peaches are among the susceptible species; air concentrations as low as a few parts per billion cause detectable injury.

First identified by the reduction in experimental rat caries (94, 95), Dean's (96) classical studies of 12 to 14-year-old children in 23 American communities established the relation between the fluoride content of the drinking water and dental caries reduction. With little or no fluoride in the water, the average child had six to nine decayed, missing, or filled (DMF) permanent teeth; with 1 p.p.m. F or more in the water, the average child had three or four DMF permanent teeth. Since 1945, studies (of which the most carefully documented is that in Newburgh-Kingston) have shown conclusively that supplementing the fluoride content of a community water

TABLE I

EFFECTS OF FLUORIDE

Effect	*Amount or Concentration*	*Time*
Vegetation damage	2 p.p.b. in air	not critical
Decreased dental caries	1 p.p.m. ± in water	first 8 yrs. of life and later
Increase in mottled enamel	2 to 8 p.p.m. or more in water	first 8 to 12 yrs. of life
Absence of osteosclerosis	< 5 mg./l. of urine	5 to 10 yrs.
Osteosclerosis in 10 per cent of population	8 p.p.m. in water	lifetime
Crippling fluorosis	more than 20 mg./day in air	10 to 20 years
Weight loss, cattle	30 to 40 p.p.m. in ration	2 to 4 years
Thyroid, altered structure or function, animals	> 50 p.p.m. in ration or water	up to 4 yrs.
Growth depression, animals	> 100 p.p.m. in ration or water	up to 4 yrs.
Kidney, altered structure or function, animals	> 100 p.p.m. in water	6 months
Acute, lethal dose, man	2500 to 5000 mg. F	death in 2 to 4 hrs.

supply to raise the concentration to about 1 p.p.m. produces mathematically equivalent reductions in caries incidence in children to those in communities where the water is naturally fluoridated (97). The available evidence indicates that benefits continue into middle age (98). The mechanism by which caries is reduced is not understood. A reasonable hypothesis is based on two factors. (*a*) Fluorapatite is considerably less soluble than hydroxylapatite, the normal bone mineral. The surface layer of enamel contains more fluoride than the internal part of the enamel, hence the defenses of the tooth are strengthened. (*b*) Fluoride inhibits acid production by certain bacteria. Perhaps those bacteria lying in immediate contact with the tooth surface and forming acid find themselves in a milieu of markedly elevated local fluoride concentration; in this way the attacking forces are weakened. The safety of water fluoridation is demonstrated by the remarkable pediatric study in Newburgh-Kingston in which no differences in the children's health in the two cities could be detected. One of the principal guarantees of the safety of water fluoridation is the large number of people (over 3,000,000 in the United States) who consume naturally fluoridated water and have done so for many years. No obvious interference with health is known; regrettably, only fragmentary epidemiological evidence sustains this clinical opinion.

The most delicate untoward effect produced in human is mottled enamel. When enamel is forming in the tooth bud the specialized cells (ameloblasts) can be injured by a variety of conditions, such as vitamin deficiency, disease, mechanical injury, or by chemicals, e.g., fluoride. Failure of the proper function of the ameloblasts causes metaplasia or hypoplasic changes which, when they involve the enamel surface, are described as mottled enamel. In mild degree, this is a "normal" occurrence. Surveys have shown that about one of five individuals reared on drinking water containing no fluoride exhibits some white spots of the enamel surface classed as mottled enamel. Note that this is a developmental defect and can only occur during the first eight to 12 years of life when enamel is being formed. Excess fluorides ingested thereafter cannot produce mottling. Teeth of persons drinking water containing about 1 p.p.m. have the same incidence of mottling as is found when the water contains essentially no fluoride. The term "mottled enamel" connotes, however, an unesthetic staining; with increasing concentrations of fluoride, increasing numbers of children exhibit milky areas, brown spots, and even irregularities of the surfaces (pits and grooves). Perhaps one child in a thousand, for example, drinking water containing 2 p.p.m. fluoride exhibits a brown spot, and when water contains about 4 p.p.m. fluoride, about one child in three exhibits some stained surfaces on some teeth. The term mottled enamel thus is used to describe both the "normal" appearance when the drinking water contains little or no fluoride and the disfiguring staining when water contains large excesses of fluoride. For water fluoridation, the important point is that fluoride at 1 p.p.m. does not alter the normal appearance of the teeth.

With continuing exposures to concentrations of fluoride 8 p.p.m. and higher hypercalcification of the skeleton (osteosclerosis) appears. Whether this reveals an increased calcification tendency or a decreased osteoclastic removal of bone is not known. The first bones to show this effect are frequently the pelvic vertebrae. Osteosclerosis is asymptomatic and no interference with the normal functions of the skeleton either structurally or as a mineral reservoir have been detected. A diligent search has failed to reveal any connection between fluoride and arthritis (99). Demonstrable osteosclerosis in man or animal bone does not develop until the fluoride concentration exceeds a limit of about 5000 p.p.m. (100, 101). Osteosclerosis does not develop in industrial workmen if their urine contains less than 5 mg. F/1. Such a concentration must be close to the noneffective limit; about 10 per cent of the residents of Bartlett, Texas, who drank water containing 8 p.p.m. fluoride showed osteosclerosis (102).

A rare industrial disorder called crippling fluorosis was characterized in the classic study of Roholm (103). Excessive exposures, certainly to more than 20 mg. fluoride a day and perhaps to 80 or 100 mg./day or more, will produce in 10 to 20 years the signs and symptoms of this disease identified by (*a*) osteosclerosis (and also loci of hypocalcification), (*b*) calcification of the broad ligaments, e.g., those along the spinous processes, which produces "poker back," (*c*) stiff joints, and (*d*) outgrowths from the surfaces of bone called exostoses. This slowly progressive disease should never be seen in the future. Excellent methods are available for the analysis of urinary fluoride which will give evidence of excessive fluoride exposure that might ultimately lead to crippling fluorosis. The overexposure can be quantitatively identified and industrial hygiene measures instituted to prevent the progress of the disease.

Growth depression appears in young animals of a number of species when diets or water contain 100 p.p.m. F or more (104). Retardation of growth has never been reported in the human. Somewhat lesser amounts of fluoride in the rations of animals produce weight loss, for example, in cattle fed 30 to 40 p.p.m. in the ration for three or four years, evidence of debility appeared.

Because fluoride is one of the halogens, and because large doses of fluoride given to experimental animals in a century-old experiment produced swellings in the neck, questions of a relation between fluoride and the thyroid have been repeatedly raised. Detectable amounts of radiofluorine (F^{18}) are found in the thyroid after an intravenous dose; however, the concentrations are parallel to those in the blood and uniformly less than the blood concentrations. Fluoride is not stored in the thyroid; the normal thyroid like other soft tissues contains only a few p.p.m. fluoride. Large doses of fluoride can produce changes in the structure and in the function of the thyroid; more than 50 p.p.m. in the food or water are required (101). A determined effort has been made to find any relation (*a*) between fluoride and goiter, or (*b*) between the severity of fluorosis and iodine deficiency in regions where

this deficiency is common. No relations have been discovered despite careful study.

Fluoride is excreted from the body almost entirely via the kidney. A small percentage is excreted in the gut via the feces. The urinary excretion is rapid. The clearance of fluoride is greater than that of chloride, not from more rapid filtration but because fluoride resorption is conspicuously less efficient than chloride. With a normal water load, fluoride resorption may be only 92 per cent as contrasted with greater than 99 per cent for chloride. The very large volume of blood passing through the kidney and the reduced efficiency of resorption accounts for the very rapid excretion of fluoride (105, 106). In advanced kidney disease with reduced urinary function, fluoride is retained longer in the body; greater percentages of the daily intake are consequently deposited in the skeleton (107). Large doses of fluoride, e.g., 100 p.p.m. in the water or more (108), have produced in experimental animals alterations in structure and function of the kidney. The question raised by Ramseyer *et al.* (109), whether 1 to 10 p.p.m. of fluoride in the drinking water produced kidney pathology in a few rats, has been resolved by a more recent study from the same laboratory. Larger groups of rats after long exposure were examined by pathologists independently in three laboratories. Opinions were unanimous that drinking water containing 1 to 10 p.p.m. of fluoride produced no kidney injury. The renal pathology described by Ramseyer *et al.* was the typical kidney changes of infection and old age.

Large doses of fluoride taken orally can produce death. The wide use of sodium fluoride as a household insecticide for many years and the innocuous appearance of the white powder so easily mistaken for powdered sugar, starch, or baking powder have led to many tragic accidents. The lethal dose in the adult human is probably 2500 to 5000 mg. of fluoride (equivalent to 5 to 10 gm. of sodium fluoride). Acute poisoning follows a very rapid course: nausea and vomiting, weakness and collapse, with evidence of circulatory failure, and death in two to four hours. Prompt antidotal procedures are useful and may be lifesaving (110). Because fluoride inhibits the action of many enzymes when the concentrations are sufficiently elevated, this is probably an enzymatic metabolic death. The precise mechanisms are not known.

From a public health standpoint, water fluoridation constitutes a new development. Here a public utility is being employed to treat and improve the health, particularly of the young. Courts have repeatedly held that water fluoridation contributes to the health of young people and is in fact a public health measure. Pleas that water fluoridation violates the constitutional guarantees of religious freedom for those who object to the use of medicines, or violates the constitutional freedom of choice because people are compelled to drink fluoridated water whether they want to or not, have been answered by the general proposition that people may be required to do or not to do certain things for the public good.

Beryllium

A surging interest in the use of beryllium as a construction material in space vehicles and perhaps as a fuel focuses increasing attention on the physiological properties of beryllium. The toxicology of beryllium in recent years has a turbulent history. A very active debate on the question, "Is beryllium toxic?" preceded the identification of the first cases of acute beryllium poisoning (pneumonitis). How such apparently infinitesimal traces of beryllium (in the air or in the tissues) could be responsible for certain cases of illness still occasions argument. Today, however, there is no doubt that beryllium is a dangerously poisonous element (111). In human patients acute pneumonitis, chronic granulomatosis often with widespread pulmonary involvement, subcutaneous granulomas, and contact dermatitis have been attributed to beryllium exposures. In experimental animals, beryllium causes cancer (lung cancer in rats; bone cancer in rabbits) and stimulates a marked hypercalcification of bone. Whether beryllium poisoning is a systemic or localized disease, whether criteria of allergy are satisfied, or whether there is a dose-response relation are fundamental and undecided points (112). At the heart of the immediate problem lies the example of the Atomic Energy Commission which set a maximal allowable concentration of 2 μg. of beryllium per cubic meter of air, a widely accepted industrial hygiene standard. After 10 years experience with this concentration as a "target figure," no cases of beryllium poisoning have appeared. This constitutes no guarantee that none will appear; as much as 16 years lag has been recorded between exposure and the development of the disabling and frequently fatal chronic granulomatosis. Enforcing rigidly maximum allowable concentration of 2 μg. Be/m.^{3} would be expensive, in some cases too expensive to tolerate. The problem is whether this figure is too stringent and overly protective.

Lead

Interest in lead poisoning continues to be high, both as a result of industrial exposure (witness the steady flow of papers from European and Russian literature) and as a childhood disease in which diagnosis is frequently difficult (113). Diagnostic procedures involving (*a*) the elevation of serum transaminase (114), (*b*) increases in the urinary (115, 116) or in the fecal excretion (117) of lead following administration of ethylenediaminetetraacetic acid tetrasodium salt (EDTA), (*c*) studies of urinary porphyrin mixtures (118), or (*d*) the elevation of urinary amino levulinic acid levels (119) may offer assistance in clarifying a puzzling picture. Studies of the mechanisms of lead effects, such as those on red blood cells by Clarkson & Kench (120) or on heme synthesis by Tishkoff *et al.* (119), may ultimately lead to better diagnostic procedures. In an effort to mobilize the lead in the soft tissues, the time-honored practices of inducing systemic acidosis or of administering parathyroid hormone have been supplanted by such drugs as EDTA or penicillamine (121) or perhaps by 2,3-dimercapto-

propanol (122). The importance of evaluating the renal status before treatment has been stressed by Fried *et al.* (123).

The requirements in the space age to provide safe living conditions for men who will breathe atmospheres 24 hours a day, seven days a week, rather than the traditional 40-hour work week, have opened a new chapter in the study of lead. A reduction of the maximum allowable concentration to one-fourth (40/168) the present industrial standard will be unacceptable on the basis of human metabolism studies by Kehoe and co-workers (124). With intermittent oral administration of lead (simulating a 40-hour week), the lead content of the body increases to an apparent maximum. In contrast, with daily exposures to abnormal quantities, lead has accumulated in the body in a linear fashion for a period of five years. This dramatically emphasizes the need to reinvestigate the problem of controlling lead poisoning to guarantee safe concentrations for continuous exposures. This same uncertainty may be found for other substances, and as a result revolutionary new industrial hygiene practices may well emerge.

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TOXICOLOGY OF ORGANIC COMPOUNDS OF INDUSTRIAL IMPORTANCE[1]

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The toxic effects of practically all the organic substances used in industry have been repeatedly stressed in the literature for many years, and their external manifestations in the form of symptoms and signs of injury are well known. It is only during the last 30 or 40 years that attention has been focused more closely on the fundamental biochemical processes which underlie these effects, and which are believed to account for the widely varying results of exposure to different compounds, even those belonging to the same chemical group. These biochemical processes involve all the dynamic physicochemical systems of the living cell and, particularly, the intracellular enzymes which catalyze its reactions. Most of these enzymes are contained in the mitochondria within the cytoplasm of the cell. The electron microscope has revealed their highly complex structure, and the isolation of the enzymes has been accomplished *in vitro* especially by the method of differential centrifugalization [Schneider (130)]. Although the enormous amount of recent research has thus succeeded in unravelling the details of the *in vitro* structure and organization of these vital constituents of the cell, full elucidation of their physiological function *in vivo* has not yet been achieved.

The knowledge which is available has, nevertheless, shed a vivid light on the relation between the toxic action of certain organic compounds and their passage as foreign substances through the body, their elimination, partial retention, detoxication, and, particularly, their interrelationship with the enzyme systems. Any injury to the mitochondria, or any inhibition of any of the groups of enzymes, can bring about a disturbance of the whole organism leading, in severe intoxication, to death, and in less severe cases to symptoms characteristic of the site of action of the toxic metabolites formed in the body.

Among the inhibitory substances, an important member, in view of some recent investigations on the metabolism of carbon disulphide (see p. 426), was tetraethyldithiocarbamyl disulphide, derived from sodium diethyldithiocarbamate by an oxidation catalyzed by the oxidase cytochrome system.

[1] The survey of the literature pertaining to this review was concluded in April, 1960.

[2] Abbreviations used in this chapter include: DNP (dinitrophenol); DPN (diphosphorydine nucleotide); SGOT (serum glutamic oxalacetic transaminase); TCA (trichloracetic acid); TCE (trichoroethanol).

Since then, many other enzyme systems have been identified and isolated, and their importance in the nature and structure of the cell itself elucidated. A key system is the Krebs citric acid cycle (82), which catalyzes the synthesis of citric acid and, subsequently, its conversion to oxalacetic acid and the generation of energy-rich phosphate bonds. Many enzymes require high-energy phosphate, such as adenosine triphosphate, for their processes of oxidation and synthesis. The synthesis of the vital ribonucleic acid from ATP is now known to be an enzymatic reaction catalyzed by specific enzymes, some of which require the presence of bivalent metallic ions, especially magnesium [Lehman *et al.* (90)]. The phosphorylation of adenosine monophosphate is one of the chief functions of the mitochondria, and the synthesis of ribose- and diribosenucleic acid is a process on which the whole metabolism of the cell depends. Oxidative phosphorylation is, in fact, the mechanism by which the esterification of inorganic phosphate provides the major part of ATP, regarded as the primary source of energy for cellular function. The statement by Kalckar (74) in 1941 that "the classification of the coupling between triosephosphate and the uptake of inorganic phosphate represents one of the greatest advances in modern biology" still remains undisputed.

In addition to the nucleotides, linked together by organic phosphorus bonds, there are also coenzymes, so called because until recently they were believed to be tightly bound to an enzyme and inseparable from it by processes of enzyme purification. It is now known that they can function as catalysts and also, more frequently, as substrates in metabolic reactions, being transformed and then regenerated through a complex cyclic reaction [Strominger (145)].

Electron microscopy demonstration of certain enzymes bound to the mitochondria has succeeded in explaining to some extent how the very complex structure of the mitochondrion can integrate the numerous enzymatic reactions required of this extremely minute body, and how the enzymes exist as multienzyme systems, chains acting together as a team in the cycle of metabolic activity. But the extreme complexity of interrelationships between nuclear fractions, supernatant fluid, mitochondrial fractions, and microsomes is still not completely solved. Nevertheless, the investigations already carried out have undoubtedly shed much light on the mechanisms by which toxic foreign substances, during their passage through the body, can exert their toxic action by producing metabolites injurious to mitochondria or by inhibiting the enzymatic reactions which can produce nontoxic metabolites.

A special mechanism for dealing with exogenous toxins appears to exist in the liver, whose cells are rich in mitochondria. These mitochondria contain a number of enzymes specifically able to deal with foreign substances. Some observers have noted congestion of the liver in animals following exposure to solvents with no known specific toxic effect on the liver and have regarded this as a manifestation of its detoxicating capacity.

The reactions of foreign compounds in their progress through the body have been described as occurring in two stages: first, the toxic including oxidation, reduction, hydrolysis, or a combination of these; and, second, the nontoxic during which conjugation takes place, including the synthesis of substances such as ethereal sulphates, glucuronides, or hippuric acid. These can be excreted without causing toxic effects. If conjugation is adequate and sufficiently rapid, the toxic substance will be completely "detoxicated"; if it is slower than that of the oxidation phase, the toxic metabolites will be left free to exert their harmful action on the living cell and especially on the enzymes contained in it [Williams (157)]. The appearance of symptoms of intoxication depends largly upon the degree of detoxication; if this fails completely and the toxic phase exerts its full effect, the whole metabolic and enzymatic activity of the cell will be disturbed, resulting possibly in death of the whole organism.

This brief review of some of the factors involved in the potential toxicity of certain toxic organic compounds helps to explain the differences in intensity and character caused by members of different, or even of the same, chemical groups. Depending on the amount of retention in the tissues, on the production of metabolites of less or greater toxicity than the original substance, and on their attack on different types of cell and different enzymes, the symptoms produced will differ in kind, in intensity, and in time of appearance.

The Aromatic Hydrocarbons

Among the aromatic hydrocarbons there is an outstanding difference in the nature of the toxic effects of benzene, toluene, and xylene, all members of the same chemical group. With high short-term exposures all three are narcotic; benzene the least so. With chronic exposure, benzene is the only member with a specific effect on hemopoietic tissue. Neither toluene nor xylene, when pure, produces the syndrome of anemia, leucopenia, and thrombocytopenia characteristic of early benzene absorption, or a final aplastic anemia. When such a benzene-like blood picture does occur during chronic exposure to toluene or xylene, it has always been, in the author's experience, associated with the use of a commercial variety of these solvents which has contained from 7 to 15 per cent of benzene (146).

The reason for this specific hemopoietic effect of benzene lies in its metabolic transformation in the body. During the phase of oxidation, initiated by various enzymes including peroxidase, catalase, and indophenoloxidase [Gabor (47)], certain phenol metabolites—phenol, quinol, pyrocatechol, and hydroxyquinol—are produced, together with the minor metabolites, muconic and mercapturic acid [Williams (157)]. These, in conditions of complete detoxication, would become conjugated with sulphuric or glucuronic acid and appear in the urine as ethereal sulphates. In fact, the measure of the change in proportion of inorganic to organic urinary sulphate can be taken as a measure of the absorption of benzene, though not of its

toxic action. It is when the process of conjugation is inadequate or delayed that some of the phenol metabolites are left free to exert their toxic action. They are mitotic poisons and have a special predilection for cells such as those of the bone marrow that are actively proliferating.

A corollary to the hypothesis that disturbance of enzymes controlling the oxidation of benezene is its fundamental toxic effect has been described by Gabor in 1959 with an attempt to use the alteration in the blood level of certain enzymes as an early indication of chronic benzene poisoning. The enzymes investigated were peroxidase and catalase, which were present in the blood of rats exposed to inhalation of varying concentrations of benzene for three hours a day on alternate days over a period of 165 days. For the first 130 days the concentration was 0.1 mg. per liter, for the next 28 days 1.1 mg. per liter, and for the last seven days 2.5 mg. per liter. The peroxidase level increased during the first 42 days to a concentration 30 per cent greater than the original and maintained this level up to 126 days, then abruptly decreased to a level of 65 per cent below the original. In the same conditions, the catalase showed a decrease from the beginning, though with periods of recuperation when it reached normal levels temporarily, only to fall again, reaching a final level 50 per cent below the original. The temporary rises were regarded as evidence of a compensatory reaction, possibly a "detoxicating" effort of the liver in the production of more catalase. The indophenol oxidase showed much less disturbance, decreasing slightly in comparison with nonexposed animals only towards the end of the experiment. Blood samples taken at intervals during the period of exposure showed oscillations of the white and red corpuscles above and below the normal, with no notable change in the hemoglobin level.

These results, particularly the rise in activity of peroxidase at the same time as a fall in catalase, followed by a simultaneous fall in both when the concentration of benezene was increased, are interpreted as indications that the first reaction represents a functional disturbance, possibly the result of a stimulative action by the free phenol metabolites on the production of peroxidase; the second reaction may represent the exhaustion of the possibility of detoxication with the appearance of morphological changes. The indophenol oxidase, in which the alteration is slower and less marked than in the other two enzymes, may be regarded as an index of the increase in the blood of pyruvic acid resulting from disturbance of oxidation-reduction processes. This acid has been stated to have an inhibitory effect on indophenol oxidase [Mikhlin (104)]. The early measurement of enzymatic activity may, therefore, be a means of estimating injury from exposure to benzene before the classical signs of poisoning appear.

Toluene and xylene.—The failure of toluene and xylene to carry out the typical benzene attack on the bone marrow is attributable to the fact that their metabolites are not the toxic phenols [Porteous & Williams (118)]. Toluene is oxidized chiefly by way of its methyl group which is replaced by a —COOH group giving benzoic acid. This is conjugated with glycollic

or aminoacetic acid to form hippuric acid. (This conjugation is normally carried out in herbivorous animals as a blockage of COOH groups and is catalyzed by coenzyme A [Adler-Herzmark (2); Chantrenne (28)]. In carnivorous animals benzoic acid undergoes a different form of conjugation with glucuronic acid, giving benzoyl-glucuronic acid.) In man, it has been estimated that although an average amount of 53.3 per cent of toluene inhaled is retained by the body, 80 per cent of this is transformed into benzoic acid and eliminated as hippuric acid within 24 hours, with about 10 to 20 per cent in the form of benzoylglucuronic acid [Srbova & Teisinger (139)]. The amount of hippuric acid in the urine which corresponds to a human exposure of 200 p.p.m. has been estimated as 3 gm. per liter [Elkins (42)], and that of benzoic acid as 2.1 gm. [Teisinger & Srbova (148)].

Though benzoic acid has not definitely been proved to be toxic per se, it may be pointed out that this amount involves, during its conjugation with glycocoll to form hippuric acid, a considerable depletion of an essential amino acid. Some observers have reported enlargement of the liver at this level of exposure, but, as already mentioned, signs of congestion of the liver following exposure to solvents with no known hepatoxic action have been recognized as evidence of its performance as a detoxicating agent.

Undoubtedly, toluene, in addition to its acute narcotic effect, does cause symptoms indicating systemic disturbance of the central nervous system—fatigue, nausea, drowsiness, paraesthesia, in-co-ordination, and insomnia—but practically all observers are agreed that when pure, it does not produce the hemopoietic syndrome characterisitc of benzene, either in animals or human beings [Gerarde (50); Browning (unpublished observations)].

Xylene of the commercial variety is a mixture of the ortho-, para-, and meta-isomers (88), the *m*-isomer usually contributing 75 to 80 per cent, and, as already mentioned, the commercial xylene may contain considerable amounts of benzene. Although chronic exposure to xylene may produce some gastrointestinal disturbance, especially nausea, the pure compound does not cause the hemopoietic effect characteristic of benzene absorption.

The chief metabolite of *o*-xylene in animals is *o*-toluic acid, excreted partly as such and partly as the corresponding glucuronide which is assumed [Bray *et al.* (18, 19)] to be *o*-toluyl glucuronide. Only a very small proportion (0.3 per cent) of the *o*-toluic acid is excreted as the glycine conjugate, *o*-toluric acid. *o*-Xylene is also hydroxylated to some extent, and the metabolites so formed may be both xylenols and hydroxytoluic acids. Bray *et al.* (18, 19) have suggested that 15 to 20 per cent of the dose may be excreted as ether glucuronides, which might be derivatives of these metabolites, since they found in the urine or rabbits given *o*-xylene, 6 per cent of the dose in the form of ethereal sulphates.

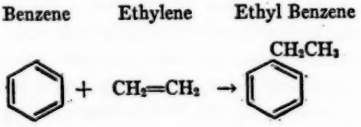
The *m*- and *p*-xylenes are more highly oxidized (80 to 90 per cent) to the corresponding toluic acids than is the *o*-isomer, and these are excreted almost entirely as glycine conjugates with practically no ester glucuronide formation. There is also a small amount of hydroxylation to xylenols; that

corresponding to *p*-xylene has been identified as 2,5-dimethylphenol.

Alkyl benzenes.—The alkyl benzenes most commonly used in industry—ethyl, propyl, butyl, triethyl, and trimethyl benzene (mesitylene)—are also devoid of hemopoietic effect. They are used as solvents for paints, dyes, inks, and lacquers, as pesticides, in protective coatings and resins, as well as constituents of automobile fuels, and as starting materials in the chemical industry.

All alkyl benzenes are irritants to skin and mucous membranes and cause increased dilatation and permeability of the capillaries that, in some cases, lead to hemorrhage into the surrounding tissues. These changes have been observed chiefly in the lungs of animals, where the most consistent post-mortem finding is a chemical pneumonitis with pulmonary edema and hemorrhage [Gerarde (50)]. They have also caused symptoms indicating depression of the central nervous system, in contrast to the initial excitatory and convulsant action of acute benzene poisoning. This depressant action is reversible when the hydrocarbons are eliminated, except in the case of one compound, *p-tert.* butyl toluene. This is a highly neuro-toxic compound causing paralysis in animals; its action is believed to be due to secondary hemorrhage in the grey matter of the cervical and thoracic spinal cord [Hine, Unger & Anderson (70)].

None of the alkyl benzenes, if pure, have a myelotoxic effect. It appears that any change in the benzene ring, whether hydrogenation, alkylation, or sulphonation, destroys the specific myelotoxicity of the benzene molecule [Scott, Cartwright & Wintrobe (133)]. Alkylation of benzene in this group is achieved by the addition of an alkyl group during the process of obtaining them from petroleum with the use of a catalyst, e.g.,



Gerarde has indeed urged that the name "alkyl benzenes," with its implied similarity to benzene, should be changed to "phenyl alkanes," which toxicologically they are.

When inhaled, these compounds are for the greater part eliminated unchanged by the lungs, and the small amount metabolized has a common pathway. The first phase of oxidation takes place on the carbon molecules of the side chains, forming alcohols or carboxylic acids. These are conjugated with glycine or glucuronic acid and excreted in the urine. These transformations are probably effected chiefly by the microsomes of the liver, though other tissues, such as the brain, spinal cord, bone marrow, kidney, and adrenal, may play a part. They differ from benzene not only in their innocuousness to the blood-forming organs with chronic exposure and their depressant rather than convulsive acute toxicity, but also in their narcotic

potency within their own group. This decreases with their chain length, falling off at four carbon atoms and diminishing steadily thereafter. The speed of onset of depression is related to the rate of absorption and transfer to the brain. Absorption depends on the water solubility, which in its turn diminishes rapidly with chain length, and multiplicity of alkylation. Thus, toluene and ethyl benzene are fast acting; *n*-propyl and *n*-butyl benzene are slow acting. The duration of the central nervous depression increases with length and branching of the side chain; this is probably related to the rate of removal of the hydrocarbon from the cells in which it is temporarily retained. Oxidation is slower with the branched side chain than the straight chain with the same number of carbon atoms. Thus, cumene and *n*-butyl benzene have a more prolonged narcotic effect than toluene and ethyl benzene. Gerarde believes that the narcotic effect of the alkyl benzenes is not structurally specific like those of the alcohols, esters, ketones, and ethers, but physical, the quality and intensity depending on the number of molecules present in the cell at the particular moment, rather than on their type. This would explain the rapid reversibility of the narcosis unless the dose is so heavy as to cause permanent injury to the brain from lack of oxygenation during prolonged unconsciousness.

The metabolism of the alkyl benzenes in general is that of hydroxylation to form carbinols which may then be conjugated with glucuronic acid and excreted as such, or further oxidized to yield benzoic or phenyl acetic acid, or of ring oxidation to phenyl fatty acids which are then further oxidized to these substances. These processes differ with the different compounds. Styrene, for example, which is more toxic than ethyl benzene, differs from it in not being converted to phenaceturic acid, though both give rise to phenyl carbonyls which are the precursors of hippuric and mandelic acids. *p*-Cymene (*p*-isopropyl toluene) apparently undergoes no hydroxylation of the ring, but oxidation only of its methyl group, giving cumic acid (*p*-isopropyl benzoic acid), and its glycine conjugate, while cumene (isopropyl benzene), is mainly converted to carbinols which are excreted as conjugated glucuronic acid, as also is *tert.*-butyl benzene.

Trimethylbenzene.—Trimethylbenzene has two isomers, pseudocumene (1-2-4-trimethylbenzene) and mesitylene (1-3-5-trimethylbenzene), of which the latter is the more widely used in industry as a thinner known as "Fleet X" which contains more than 80 per cent of it. Workers using this thinner at concentrations of 10 to 60 p.p.m. have shown some central nervous disturbance in the form of lassitude, giddiness, headache, and drowsiness [Bättig *et al.* (10)]. Animals exposed to chronic inhalation also showed some ataxia with narcosis which was reversible a few hours after cessation of exposure, but no significant variation in the blood picture. The chief metabolic product of mesitylene is dimethyl benzoic acid, which is excreted partly as the glycine conjugate, but Bättig & Grandjean found that prolonged exposure was also followed by an increased excretion of phenols, both free and bound, in the urine. Because of its resemblance to benzene in

this respect (although some blood changes found in these workers were attributed to dietary inadequacy, especially of ascorbic acid, and not to any specific hemopoietic effect of this compound), it is suggested that mesitylene cannot be regarded as entirely innocuous.

The Chlorobenzenes

Some of the chlorinated benzenes, such as *p*-dichlorobenzene and the tri-tetra- and hexa- compounds, are solids, but monochlorobenzene and *o*-dichlorobenzene are liquids and are used as industrial solvents (the former for ethyl cellulose, oils, and fats; the latter for resins and lacquers and in paint and varnish removers).

The toxicity of the chlorobenzenes is that of a central nervous system poison, and the degree of acute toxicity increases with the substitution of the chlorine atom in the benzene ring up to two and, thereafter, decreases. Thus, the maximum allowable concentration for monochlorobenzene is 75 p.p.m., and for *o*-dichlorobenzene 50 p.p.m. Above the di-compounds, the more chlorine the halogenated benzene compounds contain, the less readily are they metabolized. Thus, trichloro- and pentachlorobenzene are only slightly altered in the body, and hexachlorobenzene, used as a seed fungicide, appears to be metabolically inert [Parke & Williams (115)]. None of these three compounds form conjugated glucuronic acids, ethereal sulphates, or mercapturic acid.

Monochlorobenzene.—Although there have been no reports of monochlorobenzene causing any cases of industrial poisoning, its central nervous toxicity has been deduced from fatal cases following ingestion when it has been found to cause cyanosis, loss of reflexes, twitching of the facial muscles, and unconsciousness [Reich (123)]. When administered to animals, about 30 per cent is eliminated by the breath, but some is oxidized to phenols, especially chlorocatechol, which are excreted chiefly in conjugation with glucuronic and sulphuric acids, and also as mercapturic acid. It is in this quantitative excretion of mercapturic acid that there lies the main difference between the metabolism of monochlorobenzene and that of benzene. Whereas monochlorobenzene produces 20 per cent of mercapturic acid, benzene produces not more than 1 per cent of the administered dose [Azouz, Parke & Williams (6)].

o-Dichlorobenzene.—Used as a fumigant and insecticide as well as a solvent, *o*-dichlorobenzene has caused no industrial symptoms other than dermatitis [Downing (37)], but in animals it has produced renal damage and liver necrosis [Cameron *et al.* (25)]. Its metabolism is qualitatively similar to that of monochlorobenzene, in that it gives rise to dichlorocatechols and phenols which are excreted after conjugation with glucuronic and sulphuric acids, but it differs quantitatively in that catechol and mercapturic acid are formed in much smaller amounts and that the excretion of these metabolites is much slower [Bray *et al.* (17); Parke & Williams (114)].

Benzyl chloride.—Also known as α-chlorotoluene, benzyl chloride is

used extensively in the manufacture of pigments, resins, and perfumes, and in intermediates in the preparation of acid dyes. Its main toxic effect is a severe irritation of mucous membranes, especially of the eyes. It has, in fact, been classified as a powerful lachrymator, and this property may account for the relative lack of reports of systemic toxic effects. It is so unpleasant that 1 p.p.m. has been suggested as the maximum allowable concentration, and it therefore provides its own warning.

Its metabolic behavior is interesting in that it differs in one respect from that of the halogenated benzene compounds described above. Whereas these eventually produce mercapturic acid through intermediate precursor metabolites (sometimes known as premercapturic acids), benzyl chloride is converted directly to this substance, and at least 10 per cent of the dose can be eliminated in the urine as benzyl mercapturic acid [Knight & Young (78)].

The Halogenated Hydrocarbons

Some of the halogenated hydrocarbons are so volatile that, though their metabolites are appreciably toxic, so much is excreted by the lungs that with some compounds they are relatively small in amount. How far the toxicity of those which attack primarily the liver and kidneys is attributable to inhibition or destruction of enzymes or to actual injury of the mitochondria is not completely clear (20).

Tetrachloroethane.—The most toxic of them all, tetrachloroethane, is no longer of wide use in industry since the prohibition of its use as a "dope" for airplane fabrics during World War I. By 1916, 70 cases of jaundice with 12 deaths had occurred as the result of inhalation of its vapor [Wilcox (156)]. It still has, however, a variety of applications as a celluloid solvent in the film industry, in the impregnation of furs and skins, and, especially in Germany and France, as a solvent for a mixture of acetyl cellulose and "essence d'orient" in the manufacture of artificial pearls. In this industry a fatal case of acute yellow atrophy of the liver occurred in 1930 [Boidin, Rouques & Albot (16)]. The liver lesion is that of severe fatty degeneration of the central zones, progressing to necrosis which is frequently fatal. Tetrachloroethane intoxication may also produce a polyneuritic syndrome, which has occurred especially in the artificial pearl industry [Feil & Heim de Balzac (45); Léri & Breitel (92)].

Only 19.6 per cent of the dose inhaled by animals is excreted by the lungs; the amount retained is excreted slowly, partly in the urine, and the liver retains traces 24 hours after the last exposure [Gasq (48)]. Its actual metabolites have not been isolated, but it has been suggested that one of them may be oxalic acid and that this may be the determining toxic factor. Increased amounts of oxalic acid were found in a sample of urine of a person poisoned by tetrachloroethane [Lilliman (93)]. It has also been suggested [Williams (157)], that if it were metabolized by dehydrochlorination consisting of the loss of hydrogen chloride (a reaction which occurs in insects treated with the chlorinated hydrocarbon insecticide, DDT), it

might yield trichloroethylene and eventually trichloracetic acid, but this substance has not yet been proved to be a metabolite of tetrachloroethane [Barret, Cunningham & Johnston (7)].

Carbon tetrachloride.—Carbon tetrachloride is very widely used as a fire extinguisher, a solvent for rubber, a grease remover, and a dry-cleaning agent. Its toxic effects have been reported mainly from the point of acute toxicity when severe damage to the liver and kidneys has been observed (140).

The liver lesion following a large dose is a centrilobular necrosis. If it is not too massive, repair may begin within three or four days and may be complete in two or three weeks (38). With chronic exposure the symptoms are chiefly gastrointestinal, with nausea and vomiting, and also nervous, with headache, drowsiness, and excessive fatigue. Jaundice is rarely present.

There is some disagreement as to the actual mechanism by which carbon tetrachloride produces its hepatotoxic effect (136). Examination of the livers of rats to which it has been administered by stomach tube has shown the presence of hydropic globules in the cells. These globules contained succinoxidase and ribonucleic acid, suggesting that they were derived from mitochondria [Christie & Judah (29)], and many authorities consider that the primary biochemical lesion is the result of a direct structural attack on the mitochondria themselves. This disrupts their function, depriving them of their ability to retain the small amount of coenzyme which they possess and, thus, disorganizing their whole enzyme action.

The measurement of oxidative phosphorylation is a sensitive indicator of mitochondrial damage, and it has been found that this is inhibited *in vivo* in the liver of rats treated with carbon tetrachloride. It was also observed [Calvert & Brody (23)] that there was a definite time factor in the biochemical changes caused by carbon tetrachloride. The morphological changes in the liver occurred before the oxidative changes could be detected. It is interesting to note that oxidative phosphorylation survived a long time and that in a recent investigation by Alexander & MacDonald (3) in sheep, there was no significant change in the level of plasma alkaline phosphatase. This may lend support to the findings of Steward & Witts (143) in 1944 that the early changes in the liver are reversible. Indeed, more recent researches by Oberling (109) have shown that there is intense mitochondrial regeneration after carbon tetrachloride intoxication. On the other hand, a somewhat ominous suggestion has emerged from the investigations of Andervont (5) in his demonstration that carbon tetrachloride has a carcinogenic action on the liver of the mouse.

Another possibility of measuring slight changes in liver cells, not demonstrable by clinical examination or by the usual tests for liver function, is that of estimation of the level of serum glutamic oxalacetic transaminase (SGOT). Transaminases are widely present in animal tissues and in blood; they catalyze an amino-acid reaction, and when cells rich in these enzymes are injured, increased quantities of transaminase are released into the blood

stream. This is especially the case with injury by hepatotoxic agents such as carbon tetrachloride, and the increase of SGOT is regarded as a highly sensitive indication of its injury of liver cells. This has been confirmed in both animals [Block & Cornish (15)] and human beings [Wroblenski & La Due (160, 161)]. In men acutely poisoned by carbon tetrachloride the rise of activity of SGOT has been particularly striking. In two such cases examined by Wroblenski & La Due, the rise in SGOT level two days before admission to hospital was up to 27,840 and 12,340 units respectively as compared with the normal range of 8 to 40. In both cases there was a return to a normal level with a week. More recent work [Beaufay *et al.* (11)] on the influence of carbon tetrachloride on the bound enzymes of the liver indicates that the hepatotoxic action may be related to the release of intracellular enzymes segregated in a group of cytoplasmic particles, the lysosomes, distinct from the mitochondria. When the membrane of these particles, which is probably of a lipoprotein nature, is injured, they release a collection of soluble hydrolytic enzymes that may play an important part in the necrosis of liver cells. This extremely careful and complex investigation also revealed a time factor in morphological and biochemical manifestations; in this case the enzyme alterations were present as much as several hours before detectable microscopical liver lesions.

Trichloroethylene.—The acute toxic effect of trichloroethylene, as manifested by its use as a surgical anesthetic, is that of a powerful narcotic which when inhaled in high concentrations rapidly produces complete unconsciousness. With chronic exposure there is also some evidence of its predominant effect on the nervous system; it has been reported to cause vague nervous symptoms—headache, drowsiness, giddiness, fatigue, insomnia, and intolerance to alcohol (110). Whether it has a chronic toxic effect on the internal organs, especially the liver as in the case of carbon tetrachloride, has been much debated. On the whole, the consensus of opinion is that it has no such hepatotoxic action, but there has recently been one report from Italy [Capellini & Grisler (26)] suggesting that it may have caused slight functional disturbance of the liver. Among 12 women chronically exposed, some showed, in addition to dyspeptic symptoms, slight enlargement of the liver and an increased level of bilirubin in the blood. Estimations of the serum transaminase level, already described as an indication of liver injury, have, however, shown no increase such as that observed in carbon tetrachloride intoxication, and Grandjean (59) is of the opinion that it occurs only in persons with a predisposition to liver disorder.

Trichloroethylene has quite a remarkable metabolic performance. It forms two main metabolites, trichloracetic acid (TCA) and trichloroethanol (TCE), and a number of others which include urochloralic acid, monochloracetic acid, chloroform, and chloral [Marshall & Owens (101)]. Both TCA and the TCE fractions are excreted slowly and are demonstrable in the blood 15 days after exposure. The excretion of the other metabolites is also slow; they can be found in the urine two weeks after a single exposure,

and even longer with chronic exposure. It is suggested that this is caused by slow release of trichloroethylene retained in the tissues, where it may be bound to protein [Bartoniček & Souček (9)]. Acording to Rubino *et al.* (127), the chloral hydrate is the first to appear in the blood stream; the free TCE shows an initial decrease because of its glucuronic conjugation, but both fractions are recoverable from the blood for at least 15 days. TCA is the last metabolite to appear, its maximum concentration being present about 50 hours after inhalation and remaining for 15 days. The ratio of urinary elimination of TCA to TCE varies in man from 1:1.5 to 1:2, and the ratio of urinary TCA to atmospheric trichloroethylene is said to be from 6:1 in young subjects to 2:1 in older people.

A statistical analysis by Grandjean *et al.* (60) in 1955 indicated that subjective, vegetative, and neurological disturbances were more common in workers excreting an average amount of 67 to 180 mg. per liter of TCA than in those with an average excretion of 20 mg. per liter. Following the statement by Barrett & Johnston (8) in 1939 that the amount of TCA in the urine can be correlated with the amount of trichloroethylene inhaled, it was at one time thought that the best estimation of trichloroethylene exposure might be that of estimation of urinary TCA. More recent investigations of the relation between the urinary metabolities of trichloroethylene in workers in a dry-cleaning establishment in Italy [Rubino *et al.* (127)] have suggested that though urinary TCA is a good criterion of its own level in the blood, it is not strictly correlated with the amount of trichloroethylene itself, either in the blood or in the environment, and that TCE is a preferable criterion. TCE is present in the urine, both free and conjugated, in much larger amount than TCA. In both massive and chronic intoxication by trichloroethylene, 68 per cent of the metabolic excretion was represented by TCE as against 32 per cent by TCA, and there was a much closer correlation between the TCE in blood and urine with the level of trichloroethylene in the atmosphere and the blood. Also, there was a wide individual variation in the excretion of TCA, and it was considered a not wholly reliable guide to the amount inhaled [Butler (22)].

Dichloroethane.—Dichloroethane has two isomers, 1-1-, or ethylidene chloride, and 1-2- (symmetrical), or ethylene dichloride. The latter is the more important industrially, being a low-priced, efficient solvent for resins, rubber, cellulose acetate, and lacquers. It is also used in the production of ethylene glycol, as a degreaser for metal and wool, as an extraction solvent for soya bean and caffeine, and as an insecticide and fungicide. From the point of view of acute poisoning, it is a powerful narcotic and was at one time recommended as an anesthetic. Acute toxicity from its industrial use has not been frequently recorded considering its wide application, but four cases occurred in 1957 [Menschick (102)] in men painting the inside of a container. One man became unconscious 10 minutes after an attack of vomiting and on recovery of consciousness had muscle spasms and severe gastrointestinal disturbance; a second showed similar symptoms but with-

out loss of consciousness; the remaining two had a latent period of about one hour before developing gastric symptoms, giddiness, and weak heart action. Both these men showed some disturbance of liver function as evidenced by increased blood bilirubin and a positive Taka Ara reaction. Dichloroethane is generally regarded as having a much less toxic action on the liver than carbon tetrachloride, chloroform, and tetrachloroethane, but that it can affect the liver and also the kidneys is evident from fatal cases following accidental ingestion. Two such cases following ingestion of an antirheumatic external remedy containing sym. dichloroethane were reported by Weiss (153), in which both liver and kidneys showed parenchymatous lesions; two more [Morozov (105)], one fatal, occurred from swallowing several mouthfuls of a fluid which was later identified as dichloroethane, though no distinction between the two isotopes was made. In the fatal case there was toxic hepatitis and nephrosis.

In animals, undoubtedly, the liver damage caused by acute exposure to dichloroethane is less than with carbon tetrachloride [Heppel *et al.* (68)], but many develop necrosis and hemorrhage of the adrenal cortex and a temporary corneal opacity caused by infiltration of the cornea with lymphocytes and connective tissue cells. It is interesting to note that this effect was also observed in human beings as early as 1887 when its use as an anesthetic was suggested [Dubois & Roux (39)]. It is the only saturated chlorine compound of the ethane series which has this action [Hueper & Smith (69)].

Its metabolism has not been thoroughly elucidated. In animals it is mainly exhaled unchanged, and no metabolite has been definitely isolated. It has been suggested, however, that like other halogenated hydrocarbons it is dehalogenated *in vivo* by a hydrolytic enzyme occurring in the kidney, liver, and spleen, with formation of an oxidized hydrocarbon and a halogen ion. Other views are that oxalic acid may be one of its metabolites and that this may account for the difference in toxicity of the two isomers, the 1-1- form, yielding acetic acid, being allegedly less toxic than the 1-2- [Heppel (68)].

Trichloroethane.—Also known as methyl chloroform, trichloroethane has two isomers, 1-1-1- and 1-1-2-; according to Elkins (43), the latter is the more toxic. It has solvent properties similar to those of dichloroethane and is used for many similar purposes. Both isomers are narcotic. The 1-1-1- isomer was studied in detail by Adams *et al.* (1) in 1950; they found the principal toxic effect of single exposures in rats to be a depression of the central nervous system typical of an anesthetic agent. In such anesthetic dosage there was some relatively slight injury of the liver—fatty change and necrosis—and, as with carbon tetrachloride, the central portions of the lobules were more severely affected than the peripheral. With repeated dosage the liver damage was slight, and it appeared that animals could tolerate much greater exposure than to carbon tetrachloride without injury. As in the case of dichloroethane, the metabolism of trichloroethane has not been completely explored, but it is believed to be similar in its mechanism.

Perchloroethylene.—Perchloroethylene, or tetrachloroethylene, has sol-

vent properties similar to those of trichloroethylene and is used for similar purposes. Symptoms resulting from chronic exposure are also similar but have been less widely reported, probably owing to its lower volatility. It is eliminated chiefly unchanged through the lungs, but some metabolite is excreted in the urine. In 1939 Barrett & Johnston (8) stated that its composition was unknown, but according to Grandjean (59), it is trichloracetic acid, excreted slowly to the extent of about 20 per cent.

The blood alcohol content of animals and humans, even with inhalation of concentrations high enough to produce unconsciousness, is not raised [Paulus (117); Schleyer (129)].

The Alcohols

The primary aliphatic alcohols.—Methyl, ethyl, propyl, butyl, and amyl are the most widely used alcohols in industry. They all have irritant and narcotic properties, but, except for methyl alcohol which has a specific toxicity for the optic nerve and butyl alcohol which can cause an unusual form of keratitis, their systemic toxicity is not high.

Their narcotic potency when given by mouth increases with their molecular weight (111). It can also be correlated to some extent with their conjugation with glucuronic acid, the second of two reactions that the primary alcohols undergo. The first is the formation of an intermediate aldehyde followed by the production of carboxylic acid, which may be either completely oxidized to carbon dioxide, or excreted as such, or combined with glucuronic acid. There is little conjugation with the primary alcohols, not more than about 10 per cent of the dose, and depending on the nature and length of the carbon chain, with a peak of conjugation at about six carbon atoms. The sensitivity of rat-brain cortex respiration when it has been stimulated by potassium chloride also increases with the length of the carbon chain.

Beer & Quastel (12) have shown that *n*-pentanol has a much greater inhibitive potency than *n*-butanol, which in its turn is more potent than *n*-propanol and ethanol. The fact that these alcohols have little or no inhibitive effect on brain respiration unless it has been stimulated by potassium indicates that potassium is concerned with the activation of the citric-acid cycle of enzymes, and it is suggested that the site of action of the alcohols is the cell membrane rather than the mitochondria, which are respiring in media containing a high potassium ion concentration.

The enzyme concerned in the first step in the reaction is believed to be alcohol dehydrogenase, which catalyzes the transference of hydrogen from alcohol to diphosphorydine nucleotide (DPN) with the formation of acetaldehyde and reduced DPN. The results of Beer & Quastel (12) in their investigation of the effects of acetaldehyde on brain mitochondrial respiration have indicated that DPN has an alleviating action on the inhibitory effect of acetaldehyde and accelerates the removal of this from the system, either by oxidation or dismutation. Acetaldehyde is, for equivalent concentrations,

about 200 times more effective than ethanol in bringing about a suppression of potassium-stimulated brain respiration. There is still some controversy as to whether alcohol dehydrogenase is the enzyme of primary importance in the complete oxidation of ethanol; catalase has been suggested as a secondary mechanism [Gillespie & Lucas (53)]. Beer & Quastel state that their results do not support the view that ethanol exercises its inhibitive effect on brain respiration by preliminary conversion in the brain tissue to acetaldehyde, or that an active alcohol dehydrogenase exists in the brain.

Correlation of the toxic action of the primary alcohols with their metabolic fate is best exemplified in the difference between ethyl and methyl alcohol. Ethyl alcohol is metabolized chiefly in the liver; other organs play a subordinate part. As already described, its oxidation takes place in steps, acetaldehyde being an intermediate metabolite. This is rapidly oxidized in the organism to acetic acid (14), and in normal conditions is further oxidized to carbon dioxide and water. This reaction is not specific for ethyl

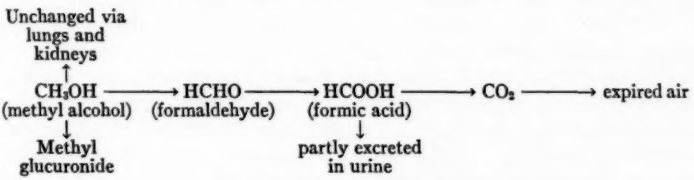


FIG. 1. Metabolism of methyl alcohol[3]

alcohol but occurs also with propyl, butyl, and amyl alcohol. Methyl alcohol, on the other hand, is oxidized in its first step to formaldehyde and then to formic acid (Fig. 1). This is believed to be effected more probably by the catalase than the dehydrogenase mechanism, and four to five times more slowly than ethanol [Keeser & Vincke (75)]. The characteristic toxic action of methanol is, therefore, believed to be attributable to the high toxicity of these metabolites and to the slowness of their elimination. Formaldehyde has been detected in the aqueous and vitreous humor of the eyes of rabbits poisoned with methanol, and formaldehyde is extremely active in inhibiting oxygen uptake and carbon dioxide production [Leaf & Zatman (89); Potts & Johnson (119)].

The site of this inhibition of retinal glycolysis is, possibly, the disturbance of phosphorylation. It appears that the primary retinal changes are followed by lesions of the optic nerve, ultimately developing into optic atrophy. In nearly all cases such optic atrophy has followed ingestion of methyl alcohol, but some have been reported following inhalation of wood alcohol [Robinson (125)]. Few investigations of the effects of chronic industrial exposure to the vapor of methyl alcohol have been made. One of these by Leaf & Zatman (89) was carried out in a factory where the air

[3] After Williams (157).

concentrations in a methanol synthesis, distillation, and stripping plant were analyzed. In the synthesis plant they were very low, less than 5 p.p.m. In the distillation plant in the late afternoon the level reached 64 p.p.m., and in the stripping plant in the early afternoon a maximum of 116 p.p.m.

The question whether ethyl alcohol, when given at the same time as methyl alcohol, can reduce the toxicity of the latter has been much discussed [Zatman (162)]. It is believed that ethanol may reduce the formation of formate by inhibiting the oxidation of methanol so that more is eliminated unchanged in the expired air and in the urine [Røe (126)]. This increased elimination was actually observed by Leaf & Zatman in their factory investigation, and they emphasize their belief that the enzymic removal of methanol is attributable to the competitive inhibitory effect of ethanol on the process of oxidation.

Among the other primary alcohols, *n*-propyl and *n*-butyl are the most predominant in industrial use, as solvents for gums, resins, oils, and cellulose ethers and esters. *n*-Butanol is also used extensively in the lacquer and dye industries. From the narcotic point of view, *n*-propyl alcohol is twice as potent as ethyl alcohol, and *n*-butyl even more so, but the only toxic effect reported from their industrial use is that associated with exposure to *n*-butyl alcohol. This is the development of an unusual form of keratitis described by Cogan & Grant (32) in 1945. It occurred in workers employed in applying a synthetic resin dissolved in *n*-butanol to Army greatcoats. They were exposed to a concentration varying from 15 to 200 p.p.m., and their symptoms consisted of irritation of the eyes with some blurring of vision. The lesions seen under transillumination consisted of clear, translucent vacuoles in the superficial layers of the cornea, especially numerous on the central portion where in the most severe cases they numbered up to 1000. They improved, and in some cases completely resolved, within 10 days of absence from work, but recurred a few days after further exposure.

The metabolism of *n*-propanol is characterized by its oxidation at a constant rate to propionic acid, which is then presumably further oxidized to carbon dioxide and water. Very little is conjugated, only large doses producing about 1 per cent of conjugated glucuronic acid. *n*-Butanol is also rapidly oxidized, about 2 per cent of the dose being conjugated.

Secondary alcohols.—Of the secondary alcohols, the most important industrially is iso-propyl alcohol; it has a wide use as a solvent in many industries. It is nearly twice as narcotic to animals as ethyl alcohol, but causes only a moderate fatty infiltration of the liver and kidneys, and no such visual disturbance as methyl alcohol. No ill-effects have been reported from its industrial use. Its greater narcotic potency in relation to ethyl alcohol is ascribed partly to its slower rate of metabolism, and partly to the fact that while it is conjugated (to a smaller extent than the other secondary alcohols) with glucuronic acid, it is oxidized in the body chiefly to acetone, the most likely catalyst being alcohol dehydrogenase. Williams (157) remarks that since acetone is slowly converted to acetate, formate, and carbon dioxide,

it seems reasonable to conclude that iso-propanol may form these compounds.

Used only to a small extent in the lacquer industry, iso-butyl alcohol is not of great industrial importance. Like the other alcohols it is narcotic to animals (to about the same extent as *n*-butyl alcohol), but has not been recorded as causing any eye injuries in man. It is metabolized by oxidation to ethyl methyl ketone, and conjugated to *sec.*-butyl glucuronide.

With the substitution of a chlorine atom, the toxicity and narcotic activity of the alcohols increases, and, again, there is more conjugation when two or more chlorine atoms are present. Thus, whereas ethanol is largely oxidized to acetic acid, trichloroethanol, a much more active narcotic, is converted in the body, partly, to the trichloroethyl glucuronide and partly to trichloroacetic acid. Both di- and trichloroethanol produce relatively nontoxic glucuronides, in contrast to the highly toxic monochloroethanol whose toxic properties are ascribed to its formation of chloroacetic acid and its failure to conjugate to the much less toxic glucuronides [El Masri, Smith & Williams (44)].

Monochloroethanol.—Also known as ethylene chlorohydrin, monochloroethanol is a colorless, mobile liquid used chiefly in the chemical industry in the manufacture of ethylene glycol and also as a solvent. In 1927 Koelsch (80) described two fatal cases resulting from considerable exposure to this compound. One man was using it for cleaning a machine, the other as a solvent for pigments. The symptoms preceding death were nausea, vomiting, headache, and giddiness. It was nearly 20 years later that a series of cases of poisoning, two fatal and nine nonfatal, occurred during the manufacture of ethylene chlorohydrin [Goldblatt & Chiesman (55)]. In one of the fatal cases death occurred 14 hours after exposure to a high concentration of a vapor which included ethylene chlorohydrin. Here, also, the preceding symptoms were those of gastrointestinal and central nervous disturbance. In neither fatal case did autopsy result in a clear definition of the cause of death. Animal experiments, however, showed that lethal concentrations (1120 p.p.m. for rats) caused renal hemorrhage and tubal degeneration, fatty degeneration and necrosis of the liver, and high nonlethal concentrations caused inhibition of cardiac action, respiration, and smooth muscle, and nerve transmission. These toxic effects are ascribed to its metabolic characteristics, especially to the formation of monochloracetic acid which is 20 to 40 times more toxic than acetic acid or the metabolites of di- and trichloroethanol (85).

The Glycols

The glycols and their ethers and esters are used to some extent in industry as solvents for nitrocellulose and cellulose acetate and as vehicles for pharmaceutical preparations and skin lotions.

Ethylene glycol.—Best known as an antifreeze mixture for motor vehicles, ethylene glycol is not sufficiently volatile to be an industrial hazard in

normal conditions, but there has been one report from Italy [Troisi (150)] of chronic poisoning from inhalation of the vapor from a mixture containing 40 per cent of ethylene glycol, 55 per cent boric acid, and 5 per cent ammonia, heated to 105°C. The chief symptoms were recurrent attacks of unconsciousness, and some of the affected women showed nystagmus. These effects were attributed to an action of the substance on the central nervous system.

When taken internally, ethylene glycol is extremely toxic; in fatal cases death has been due to renal failure, with crystals of oxalic acid deposited in the tubules. In fact, in poisoning by any of the glycols or their derivatives the outstanding toxic effect is exerted on the kidneys. Propylene glycol is about half as acutely toxic to animals as ethylene glycol and very much less with repeated administration. Injury of the kidneys, in the form of hemorrhage and casts, has been produced by fatal intravenous dosage, but continued feeding in concentrations of 2.45 to 4.9 per cent of the food did not cause the deposition of oxalic acid stones as with ethylene glycol. For neither propylene nor butylene glycol have there been any reports of ill-effects on human beings.

Diethylene Glycol.—Used in the lacquer industry, and in face creams and as a vehicle for medicinal preparations, diethylene glycol is less toxic than ethylene glycol judging from their respective minimal fatal doses for animals by intravenous injection (ethylene glycol, 8.54 g. per kg., diethylene glycol, 14.8). But, when mixed with food, 5 to 10 per cent has proved fatal to some animals within a few weeks. Some of these animals showed stones of calcium oxalate and phosphate in the bladder. Damage to the kidneys consisted of extensive injury to the tubules, causing obstruction and uremia. The toxicity of diethylene glycol by ingestion for human beings was strikingly illustrated by a series of deaths occurring in the United States in 1937 from the use of an "elixir" of sulphanilamide, with diethylene glycol as the vehicle [Ruprecht & Nelson (128)]. The initial symptoms included nausea, dizziness, pain in the kidney region and abdomen. These were followed in the course of a few days by oliguria and anuria, with a final development of uremic coma. The most characteristic changes, severe "chemical nephrosis," were found in the kidneys [Geiling & Cannon (49)].

Dipropylene glycol.—The higher homologue of diethylene glycol, dipropylene glycol is apparently less toxic but in large doses has produced similar, though less severe, kidney damage in animals by oral and intravenous administration.

The monoalkyl ethers.—The trade names of the monoalkyl ethers are Cellosolve (ethylene glycol monoethyl ether), Methyl Cellosolve (ethylene glycol monomethyl ether), and Butyl Cellosolve (ethylene glycol monobutyl ether). They are good solvents for cellulose esters and ethers, and are widely used in lacquers. Methyl Cellosolve has also been applied to the "fused collar" industry, where the collars are dipped in a solution of Methyl

Cellosolve and finished under heat and pressure. Butyl Cellosolve is used in detergent solutions, and with phosphoric acid in metal cleaners to prepare sheet metal for lacquering and enamelling.

Ethylene glycol diethyl ether (Diethyl Cellosolve) and diethylene glycol monoethyl ether (Carbitol) are other members of this group with smiliar solvent properties and similar potential toxicity, but in varying degree, for the kidneys in animals. Their industrial hazard is not great in normal conditions.

In animals Butyl Cellosolve has proved the most toxic, and Carbitol the least, the butyl compound producing symptoms of kidney inflammation [Werner *et al.* (154)]. Methyl Cellosolve has been reported to have an effect, unusual in this series, on the central nervous system and the blood picture. This "toxic encephalopathy" associated with slight blood changes was observed in the fused collar industry. The symptoms were a change of personality from liveliness and intelligence to dullness and apathy [Greenberg *et al.* (63); Parsons & Parsons (116)]. A case of nervous disturbance, with symptoms of irritability and insomnia, following the use of a paint containing Butyl Cellosolve has recently been brought to the notice of the author (unpublished observation).

The differences in toxicity of these chemically related compounds are probably to be found in differences in their metabolic progress, depending upon the occurrence of primary, secondary, or tertiary alcoholic groups and upon the relationship of these groups to each other. Where primary alcohol groups occur, oxidation might result in the corresponding acids which may exert their toxic action by inhibiting enzyme systems. Some of them undergo conjugation with glucuronic acid, but only if they contain six carbon atoms; even then the conjugation is less than 10 per cent. Ethylene glycol, for example, is oxidized through oxalic and glycolic acid to carbon dioxide and water with practically no conjugation [Gessner, Parke & Williams (51)], whereas propylene glycol, with considerably lower toxicity, is metabolized to the extent of two-thirds of the dose to lactic acid, which is then oxidized to carbon dioxide and water through the tricarboxylic acid cycle.

Carbitol, though potentially a kidney poison to animals, probably owes its relatively low toxicity to the fact that it is excreted as a glucuronide and, also, that its lower vapor pressure limits its toxicity to exposure by inhalation. Undoubtedly, the predilection of those members of the group for the kidney as the site of their toxic action is associated with their metabolic formation of oxalic acid and its tendency to block the tubules of the kidney in their efforts to eliminate it, but it is now believed that other metabolic intermediates, particularly glyoxal, may also be involved [Doerr (36)].

Cyclohexylamine and Dicyclohexylamine

These two liquids are used in the emulsification of soaps, as intermediates in the dying industry, as solvents for dyestuffs, and as vulcanizers in

the rubber industry. Neither has proved toxic to human beings, but in animals both act as neurotoxins, the toxic action being exerted on the motor centers of the spinal column and medulla [Flinn (46)].

The lethal oral dose of cyclohexylamine has not been established; repeated doses of 100 mg. per kg. body weight were not lethal after 82 days, and at autopsy there were no lesions of internal organs. With subcutaneous injection, of 0.5 gm. per kg. convulsions, after a delay of several hours, preceded death, but injections of 0.25 gm. per kg., repeated daily for 10 days, had no ill-effects. Application to the skin resulted in dermatitis but not absorption of lethal amounts.

Dicyclohexylamine is the more toxic of the two to animals. Convulsions, temporary paralysis of the hind quarters, and death occur sooner with a smaller dose. Like cyclohexylamine, if left on the skin, dicyclohexylamine will produce dermatitis. According to Flinn, unlike cyclohexylamine, lethal amounts can be absorbed. No detailed investigations of the metabolism of these compounds have been made, but Williams (157) states that cyclohexylamine appears to be readily oxidized in the dog, with no aromatization to aniline or its metabolites.

The Nitrophenols

The nitrophenols provide a striking example of the difference in toxic action of members of the same group of compounds. Even the isomers of the mononitrophenols show this difference, since the *o*-compound is relatively innocuous, while the *p*-nitrophenol is rapidly fatal to animals [Beutner & Block(13)].

Mononitrophenols.—Mononitrophenols, of which there are three isomers, are used chiefly in the synthesis of dyestuffs. None of the isomers have any such influence on basal metabolism as that exerted by dinitrophenol, and none increase the rectal temperature, but rather depress it [Cameron (24)]. (Cameron did not, however, regard rectal temperature as a reliable index of metabolic action.) With regard to methemoglobin formation, the mononitrophenols are not as effective as some of the dinitrophenols, though small inconstant amounts are found with 2-nitrophenol [Grant (61)]. *o*-Nitrophenol is almost completely excreted in the urine of rabbits, more so than any other phenol derivative [Cameron (24)]. The main excretory product of all the isomers is a nitrophenylglucuronide, with small amounts of dihydric phenols. According to Williams (157) the relatively higher toxicity of the *p*-derivative appears to be an intrinsic property of this compound per se, since all its metabolites, including 4-nitrocatechol appear to be nontoxic.

Dinitrophenols.—There are six isomers of the dinitrophenols which are also used in the synthesis of dyestuffs in the manufacture of explosives, and as preservatives of timber.

The most important isomer, 2,4-dinitrophenol, is not a former of methemoglobin as are, to a small extent, the 2,3-, 2,5- and 3,4- isomers, but

it has a powerful stimulatory effect on the basal metabolic rate, causing a rise of temperature as high as 110°C., loss of weight, profuse perspiration, and nervous disorders. Liver and kidney damage has also been observed by some authorities. Changes in the blood cholesterol level following administration to human beings have been reported [Grant & Schube (62)], but the evidence on this point from animal experiments is conflicting [Koch, Lee & Tainter (79); Tainter *et al.* (147)].

Similarly, with regard to kidney injury, Magne, Mayer & Plantefol (99) found some evidence in chronic poisoning, but Schulte & Tainter (131) found no change in the renal function of rabbits receiving repeated subcutaneous injections of dinitrophenol for as long as 77 days. In industry, the majority of cases of dinitrophenol poisoning have occurred from handling the finished product and in the centrifuging, drying, and packing operations [Koelsch (80)], and, during World War I, in the manufacture of explosives.

Acute poisoning is usually sudden in onset, with severe fatigue, thirst, profuse perspiration, oppression of the chest, extreme nervous agitation, a rapid irregular pulse, and raised temperature. In fatal cases colic and diarrhea, muscular cramps, excitement, and convulsions may precede coma and death. In less severe cases, gastrointestinal symptoms are also prominent, and there may be tenderness over the liver and jaundice.

It has been shown that the major excretory product in dog and man is probably 2-amino-4-nitrophenol [Magne, Mayer & Plantefol (99)] and its presence in the urine, becoming or remaining high, has been used as an indication of neglect of preventive measures in factories where dinitrophenol was used [Horner (71)].

Dinitrophenol has a delayed effect, the formation of cataracts, when taken, generally in the form of the sodium salt, as a remedy for obesity. In spite of warnings, its use for this purpose became extremely popular in the years following 1933; Horner states that during the first 15 months after its introduction in that year, 100,000 persons took the drug for weight reduction. In addition to cataracts, skin lesions were a frequent result—dermatitis, urticaria, and angioneurotic oedema. Otitis media, agranulocytosis, neuritis, and cardiovascular damage were also reported. The occurrence of cataract was first described by Horner, Jones & Boardman (72) in 1935, and in the same year Cogan & Cogan (31) reviewed 20 cases which had appeared in the literature. The cataracts have characteristic features distinguishing them from the senile or traumatic variety. Their onset may be delayed for months or years after taking the drug and is usually marked by blurring of vision. The most striking change occurs in the posterior part of the lens in the form of a dense granular lustrous deposit. The opacities gradually invade the cortex and, finally, the nucleus. In the late stages the whole lens is divided into radiating sections separated by dark bands; glaucoma is a not infrequent sequela.

Efforts to reproduce the cataractogenic effect in animals have been un-

successful in ordinary laboratory animals, but have succeeded in chicks; these, when kept on a diet containing 0.1 to 2.5 per cent of dinitrophenol (DNP) develop, after four to six hours, a fine opacity of the lens progressing to a marked opacity of the anterior portion within 24 hours [Robbins (124)]. Many attempts have been made to elucidate the mechanism of the toxic action of dinitrophenol, both with regard to its stimulatory effect on metabolism and its cataractogenic action; these were fully reviewed by von Oettingen (112) in 1949. The outcome of the numerous investigations is that it is an inhibitor of phosphorylations in biological systems and that its difference from the less toxic isomers is probably based on the different aminophenols which are their main metabolites. The relative potency of the dinitrophenols in stimulating respiration is related to their structure—nitro-groups being most effective in the ortho- and least in the meta- position—except in the case of 2,6-dinitrophenol, which is unique in this series in having side chains on both sides of the hydroxyl group [Grant (61)]. This compound also fails to conform to the correlation between potency as respiratory stimulants and the degree of acidity—the more strongly acid compounds stimulate respiration more powerfully. The increase in respiration rate in rats was found to be only 5 per cent, and the increase in total volume only 15 per cent for this isomer, as compared with 16 and 37 per cent, respectively, for 2,4-dinitrophenol; 12 and 31 per cent for 2,3-dinitrophenol; and 9 and 21 per cent for 2,5-dinitrophenol.

When Cameron (24) in 1958 compared the effects on oxygen consumption in the rat of the six dinitrophenols, she found a lack of correlation between increased carbon dioxide output and oxygen uptake with some of the compounds and suggested that some of the respiratory stimulation might be due to simple hyperventilation rather than to interference with metabolic pathways. Grant does not agree with this view; he states that if the excess liberation of carbon dioxide were caused by hyperventilation, the increased carbon dioxide production would vary in strict proportion to the effect on ventilation. The fact that in his experiments there was a complete absence of such correlation suggests that the CO_2 effect is due to an action on cell respiration. Ramsay (120, 121) has further suggested that a component, known as the "exercise stimulus," in the regulation of breathing may also be involved in the hyperventilation produced by 2,4-dinitrophenol. He postulated that the site of this stimulus may be the nerve receptors in the region of the muscles, since he found that after perfusion of the limb of a dog with blood into which 2,4-dinitrophenol had been injected, an increase of the metabolism of the limb caused an increase in pulmonary ventilation. He believed, therefore, that these receptors were sensitive to changes in metabolic activity.

Investigations of the *in vitro* mechanism of phosphorylation by Clifton (30) and others indicate that dinitrophenol acts on the basic mechanism in the cell by which phosphate bond generation is coupled to oxidative reactions. Loomis & Lipmann (95) have shown that uncoupling, which can be achieved by very small concentrations of DNP with no effect on, or

with stimulation of oxidation, is reversible. A more recent investigation by Wessels (155) suggests that DNP can also catalyze the synthesis of ATP by chloroplasts, which are also able to convert light energy into the pyrophosphate-bond energy of ATP. The mechanism of this action is not yet fully explained. Wessel puts forward several alternative hypotheses; one is that this ability of DNP may bear some relation to its uncoupling action in oxidative phosphorylation. Other nitrophenols capable of this reaction included 2,5- and 2,6-dinitrophenol and *m*- and *o*-nitrophenol.

Further correlation with the effect of DNP on oxidative phosphorylation is suggested by its depressant effect on the renal secretion of uric acid in chickens, on the basis that the tubular transport of uric acid may be dependent on energy supplied by oxidative phosphorylation [Nechay & Nechay (107)].

The mechanism by which 2,4-dinitrophenol exerts its toxic action specifically upon the lens and the variation in species response have been the subject of many hypotheses, but no explanation has been experimentally verified. In view of the investigations described above, the opinion expressed by Krause (81) in 1938 would seem to approximate most closely to the present view of DNP metabolic behavior. He stated that "if the reaction of the lens is comparable to other tissues, DNP cataract arises from the interference with the creatine and adenosine processes in the lens."

Dinitro-orthocresol.—Dinitrol-orthocresol (2-methyl 4,6-dinitrophenol) is used chiefly as an insecticide and has also been used for weight reduction. There were fatal results in at least one case [Goldman & Haber (57)] and cataract formation in many others [Gilbert-Dreyfus & Onfray (52); Vannas (152)]. Industrial poisoning, with symptoms similar to those following exposure to dinitrophenol, has also been reported [Jordi (73); Schwarz (132)]. Jordi emphasized that, unlike dinitrophenol, dinitrocresol was not included in the official list of agricultural poisons in Switzerland, although it was widely used as a spray.

It is eliminated slowly; after ingestion only about 1 per cent of a single dose of 75 mg. is excreted in 24 hours, and it may take several weeks for the blood level to fall to zero. Its metabolites have been investigated in the rabbit by Smith, Smithies & Williams (134). They found that about 5 to 6 per cent of an oral, near-lethal dose is excreted as free dinitro-orthocresol, and 1 per cent in conjugation, mainly with 6-acetamido-2-methyl-6-nitrophenol, together with small amounts of 3-amino-5-5-nitro salycilic acid and conjugates of 4-amino-2-methyl-6-nitrophenol. The 6-acetamido-4-nitro-0-cresol is 20 times less toxic than dinitro-orthocresol and may, therefore, be regarded as a true detoxication product.

The Nitriles

From the industrial point of view, the most important of the nitriles, or alkyl cyanides, is acrylonitrile. It is attaining increasing importance as one of the raw materials in the manufacture of synthetic rubber and thermo-

plastic resins, as an intermediate in chemical syntheses, and in the synthetic fiber industry. Other members of the group, such as acetonitrile, are used as industrial solvents, and some, such as trichloroacetonitrile, as fumigants and insecticides.

As a group, these compounds are a good example of the relation between toxic action and metabolism, since their toxicity depends almost entirely upon whether their metabolic progress within the body is accompanied by liberation of the free cyanide ion.

Acrylonitrile.—A liquid volatile at room temperature, acrylonitrile can cause toxic symptoms whether ingested, inhaled, or absorbed through the skin. Fatal poisoning has occurred from accidental ingestion of a preparation known as Ventox, used as a fumigant, and from its application as a pediculicide [Grunske (65); Lorz (96)]. In both cases death was preceded by vomiting, cyanosis, and loss of consciousness. In the case of a child to whose feet it had been applied, it was believed to have been absorbed in lethal amounts by the skin; the symptoms were, in fact, similar to those observed in cyanide poisoning.

In industry, the hazard is primarily that of inhalation of the vapor [Malette (100)], but few serious and no fatal cases of poisoning have been recorded. (67). Subacute symptoms, following exposures varying from 16 to 100 p.p.m. and including headache, slight anemia, irritation of the skin and mucous membranes, and sometimes jaundice, were described by Wilson (158), and Wilson, Hough & McCormick (159).

Animals fatally poisoned by acrylonitrile show convulsions and cyanosis. Dogs are particularly susceptible; in the experiments of Wilson *et al.*, rats were able to survive concentrations (100 p.p.m.) which were fatal to all the dogs exposed. Histological changes observed in the brain were characteristic of anoxia, and Dudley, Sweeney & Miller (41) also observed renal injury and bronchopneumonia. Many animals are able to convert sublethal doses of cyanide to thiocyanate; thiocyanate can, on the other hand, be converted to cyanide (58). Administration of thiocyanate can, therefore, produce symptoms resembling subacute cyanide poisoning. This is an example of the reversible action of an enzyme on the metabolism of certain substances. In normal metabolism the conversion of hydrocyanic acid to thiocyanic acid is regarded as a "detoxication" mechanism, but for abnormal quantities entering the body this mechanism would most probably fail to function adequately. In fact, a rise in the level of thiocyanate in the blood of animals which did not return to normal 12 hours after exposure has been regarded as an indication that the detoxication capacity was exceeded.

The enzyme chiefly responsible for the conversion of cyanide in the body to thiocyanate is known as thiosulphate transsulphurase, or rhodanase. It is widely distributed in animal tissues, with especially high activity in the liver (86). In view of the high susceptibility of the dog to poisoning by acrylonitrile, it is interesting to note that the tissues of the dog have been found to have a considerably lower concentration of rhodanase than those

of the less susceptible rat [Brieger, Rieders & Hodes (21)]. Rhodanase catalyzes the transfer of sulphur from thiosulphate to cyanide to form thiocyanate and sulphite. In rat-liver homogenates it is localized in the mitochondrial fraction, and its activity is low unless the mitochondria are caused to swell or disrupt, when its activity increases [De Duve *et al.* (35); Greville & Chappel (64)].

Another enzyme, also a transsulphurase (β-mercaptyruvate), may also be concerned in catalyzing the transfer of sulphur to cyanide. According to the recent work of Kun & Fanshier (83, 84) this enzyme is also widely distributed in the tissues, but in rat liver it appears to be present in highest concentration in the "nuclear" and "soluble" fractions rather than in the mitochondria themselves. The soluble fractions were found to contain microsomes, and the supernatant fluid represented only one-quarter to one-half of the total enzyme content, suggesting that it is bound by the nuclei and microsomes.

It has already been seen that thiocyanate, if formed in large amounts by enzymatic action, can, owing to the reversibility of this action, become potentially toxic [Anderson & Chen (4)]. The use of sodium thiosulphate as a therapeutic measure in acrylonitrile poisoning might, therefore, appear to be theoretically inadvisable, but as Brieger, Rieders & Hodes (21) point out, the formation of high concentrations of thiocyanate is not to be expected in man. An increase of thiocyanates in the urine of dogs exposed to the vapor of acrylonitrile was found by Lawton, Sweeney & Dudley (87). It has been suggested that this might be used as a measure of poisoning by acrylonitrile, but this is not accepted by all authorities.

Methacrylonitrile.—Also used in the manufacture of synthetic rubber and other types of polymers, methacrylonitrile's toxic effects in animals are similar to those of acrylonitrile, with a typical cyanide-like action and with about the same degree of toxicity. But whereas it has a less irritative action on the skin, it is more readily absorbed by this route than is acrylonitrile [McOmie (98)].

Trichloroacetonitrile.—Known also as Tritox, trichloroacetonitrile is used as a fumigant, pest controller, and insecticide. It is extremely irritating to the mucous membranes of animals, and lethal concentrations are followed by dyspnea and convulsions. Death is primarily attributable to lung injury, acute hyperemia, and edema. With slightly lower concentrations, acute degenerative changes develop in the heart, liver, and kidneys [Treon *et al.* (149)]. The urine of animals exposed to air containing 1.33 mg. per liter of trichloroacetonitrile contained a substance that was either trichloracetonitrile or some compound which responded to the same test. Its further metabolic features do not appear to have been investigated in detail.

Carbon Disulphide

Carbon disulphide has long been used in the rubber and viscose artificial silk industries; it is also used in extraction and pharmaceutical proc-

esses, in the manufacture of waterproof cements and transparent paper, and as an insecticide for vines and tobacco plants.

Its chronic toxic effects have been more widely recognized in industry than its acute narcotic effect. In high concentrations it may cause loss of consciousness preceded by delirium or acute mania and followed by death from respiratory failure. Table 1 represents a summary of the results of an investigation in 1939 by the Department of Scientific and Industrial Research, London, England, showing the toxic effects of various concentrations. The symptoms of chronic poisoning by carbon disulphide are predominantly nervous, with peripheral effects in the form of polyneuritis, central effects consisting of a strio-pallidal, or Parkinsonian syndrome; psychic abnormality or mental derangement; and eye lesions including lesions of the optic nerve, and retina, and kerato-conjunctivitis.

TABLE I

TOXIC CONCENTRATIONS OF CARBON DISULPHIDE

Concentration of Vapor in Air		Duration of Exposure	Effect
parts by volume (approx.)	mg./l (approx.)		
1 in 300 to 1 in 500	10 to 6	About ½ hour	Serious illness and danger of mania and coma.
1 in 3000	1	Single exposure for a few hours	Severe headache and mental dullness or confusion.
		Daily exposure	Increasingly severe symptoms with neuritis, distorted vision, and mental disturbance.
1 in 15,000 to 1 in 30,000	0.2 to 0.1	Repeated daily exposure	General condition of ill health headache, drowsiness, and hysterical outbursts.

In recent years much attention has also been focused on a possible long-term effect—the development of arteriosclerosis, sometimes associated with kidney lesions, occurring at a relatively early age but with a latent period of about 20 years. In cases of this kind, described by von Rechenberg (122) in 1957, the arteriosclerosis was found especially in the arteries and arterioles of the cerebrum, but also in those of the peripheral muscles where it affected the media with calcification of this coat. Hypertensive kidney injury, with high blood pressure, some albuminuria, slight microhematuria, and a raised residual nitrogen were also observed—a condition

designated as "CS_2 glomerulosclerosis." The whole syndrome, consisting of encephalopathy, hypertensive nephropathy, and medial sclerosis (a vascular sulphocarbotoxic late syndrome), does not always present itself in the complete form. Individual cases may show only some aspects; for example, diffuse vascular encephalopathy may be present without hypertensive nephritis.

With short-term exposures, even to high concentrations, CS_2 is rapidly removed from the body on cessation of exposure and, owing to the rapid drop in the blood level, the amount retained is normally small. With continued exposure, on the other hand, although blood saturation is reached within 1½ hours with concentrations of 25 to 50 p.p.m., entrance into the body fluids and tissues is much slower; even after two days the tissues are still taking up labelled CS_2 [McKee *et al.* (97); Srittmatter, Peters & McKee (144)]. It has been shown that when CS_2 is inhaled, 10 to 30 per cent is eliminated by the lungs, about 1 per cent is excreted in the urine, and the remaining 70 to 90 per cent undergoes metabolic transformation in the body. By parenteral routes, the proportion retained is much less—20 to 50 per cent [Souček (137)].

The actual mechanism of the metabolic transformation is not completely understood, but it appears to be related to the binding of the portion retained in the tissues with substances containing a nitrogen atom—peptides, proteins, and amino acids. These new products are decomposed by proteolytic enzymes with more difficulty than normal albumen. The enzymic reactions, especially with the amino-acid compounds, produce dithiocarbamates, which can be further decomposed by the desulphydrase system of enzymes, liberating H_2S. This may, in its turn, be rapidly oxidized to H_2SO_4 and excreted in the urine as sulphate. Some observers have, indeed, stated that exposure to CS_2 is followed by an increased excretion of inorganic sulphate [Strittmatter *et al.* (144)], and it has been shown that radioactive H_2S administered to animals is promptly oxidized and eliminated. Within five to ten minutes 50 per cent of the total sulphur in the urine is in the form of inorganic sulphates, and within 30 minutes these show a considerable rise [Gunina (66)].

Possibly, the dithiocarbamates may also give rise ultimately to isothiocyanates. Dithiocarbamate acids contain relatively strong acid sulphydryl groups, and, in fact, the serum of guinea pigs exposed to inhalation of 300 to 1800 μg. per liter of CS_2 has shown an increase of 88 per cent of sulphydryl groups. They tend to be statistically higher than normal in viscose workers exposed to an average concentration of 200 μg. per liter [Souček & Madlo (138)]. The dithiocarbamate in the serum was shown to decompose under the influence of heat and H_2SO_4 giving free CS_2. These facts indicate that the intramolecular reaction of CS_2 with albumen causes a marked alteration in the molecule.

The liver is, undoubtedly, an important organ in the metabolism of CS_2 and some recent investigations in Germany have suggested that signs of its

capacity for detoxication of CS_2 are shown by changes indicating a disturbance of capillary permeability. This endothelial disturbance, present probably in the general arterial system, and generally, as already remarked, associated with premature arteriosclerosis, is not, however, directly its cause. Other factors, such as hyperlipemia, and especially an increase in blood cholesterol, have been held responsible [Michalova, Bartoniček & Zastavá (103)].

Cholesterolemia is certainly a feature of CS_2 intoxication in animals, but it is not certain whether it is directly associated with the changes in the kidney. Von Rechenberg (122) believes, rather, that the nephropathy is associated with the brain lesions and with the specific sclerosis of the arterial media in the sense of a general vascular toxic effect of CS_2 or its metabolites. Nevertheless, cholesterolemia may be another manifestation of altered body metabolism intricately bound up with the enzyme systems of the body, and particularly with the metal complexes associated with them and required as activators (94). Alkaline phosphatase, for example, requires zinc and magnesium ions as activators, and the urinary excretion of both has been found increased in animals chronically poisoned with CS_2, with a simultaneous decrease in alkaline phosphatase activity. The chain of events in this metabolic disturbance appears to be that of a chelating effect by the dithiocarbamate and thiozolidone resulting from the reaction of CS_2 with free amino groups in the body. This chelation results in the formation of nonionized complexes with the polyvalent metal ions, so that these are prevented from performing their natural function in normal cell metabolism.

An interesting aspect of this hypothesis is the way in which it can be linked with the predominant nervous lesions of CS_2 toxicity, central or peripheral. Histological examination of the nerve tissues of animals subjected to prolonged and severe exposures to CS_2 has revealed degenerative changes in the cells of the brain and spinal cord amounting to almost complete destruction and loss of function. In the brain and spinal cord, copper is normally present as a structural part of cytochrome oxidase and coenzyme A dehydrogenase. If it is unavailable, the metabolism of these nerve cells may be severely disorganized. Copper is also known to be tightly bound by the thiocarbamate groups, and in animals with severe pathological lesions of the spinal cord and cerebral cortex, the copper content of these tissues is about half the normal [Cohen *et al.* (33)]. Thus, it appears that the specific toxic action of CS_2 on the nervous system can be directly associated with its metabolic behavior.

Tetraethyl Lead

Tetraethyl lead [$Pb(C_2H_5)_4$], in the form of "ethyl fluid," a mixture of 49 to 63 per cent of tetraethyl lead with ethylene bromide or dibromide or dichloroethane, is used chiefly as an antiknock additive to motor fuels. It is very volatile—at 18°C., air saturated with its vapor contains about 5 mg. per liter. It is, therefore, most commonly absorbed by inhalation, but

Kehoe & Thaman (76) have shown that in animals it can also be absorbed by the skin.

Its chief hazard arises during its manufacture, during mixing with motor fuel, and, especially, while cleaning storage tanks which have contained leaded petrol. Kitzmiller, Cholak & Kehoe (77) state that men exposed to air in tanks in which the sludge may contain 0.1 per cent or more of tetraethyl lead may inhale and absorb a lethal dose in half an hour. In less severe conditions, in a day or a number of successive days a dose sufficient to cause a serious or even fatal illness can be absorbed. It produces a syndrome of intoxication entirely different from that of inorganic lead (27).

The symptoms, whether acute and fatal, severe but not fatal, or mild in degree, are neurotoxic in character. In acute fatal cases, delirium, mania, hallucinations, convulsions, and coma may precede death occurring within a few days of exposure. In severe but not fatal cases, there is often mental confusion, excitement, restlessness, disorientation, sometimes depression, and nausea, abdominal pain, and vomiting. Such cases frequently progress towards the more acute mental disturbance, but if the critical stage is passed within a week or two of the onset, recovery usually occurs within four to 10 weeks, though relapses are not uncommon. In mild cases, such as those described by Müller (106) in men employed in the recovery of lead scrap used for the production of tetraethyl lead, the complaints include insomnia with bad dreams, restlessness, loss of appetite, and gastrointestinal disturbance—a picture difficult to distinguish from an anxiety state. These cases usually regress rapidly on cessation of exposure.

The distribution of tetraethyl lead in the tissues following absorption differs, at least in its initial stages, from that of inorganic lead, as well as in its chief site of deposition. It has been shown that inorganic lead is very sparsely deposited in the brain of animals, even when given intravenously, and that it is not accumulated there [Ginsberg & Weatherall (54); Goldblatt & Goldblatt (56)]. In fatal tetraethyl lead poisoning, both in animals and humans, "volatile and nonvolatile" lead compounds have been found in the brain in amounts up to 12 to 21 μg. per gm., and Goldblatt has suggested that the critical level in the brain at which signs of central nervous disturbance may appear is 2 to 3 μg. per gm. He suggests, also, that the volatile organic lead compounds have a special predilection for lipoid and nerve tissue, a view also suggested by Smusin (135) on the basis of his experiments on the relation between tetraethyl lead and cholinesterase (see below). More recent investigations on its metabolism by Cremer (34) and Stevens *et al.* (141, 142) have not supported the view that the reason for its predominant effect on the nervous system is essentially its fat-soluble character allowing selective localization in nerve tissue, or that its toxicity is a function of its lead ion content. It now appears that the metabolic products of tetraethyl lead are more toxic than the original compound.

"Volatile lead" has also been identified in the liver, though without certainty that this compound was in fact tetraethyl lead [Norris & Gettler (108); Kehoe & Thaman (76); and others]. An analysis of liver tissue concentrated by low temperature vacuum distillation and examined spectroscopically, with a confirmatory dithizone analysis, has, however, shown that it was present in small amounts. During its metabolism within the body, other water-soluble organo-compounds are formed, of which those most closely investigated are triethyl and diethyl lead. It is to the former of these, triethyl lead, that the toxic effects of tetraethyl lead are attributed [Cremer (34)].

The isolation of triethyl lead was achieved by Cremer and by Stevens, Feldhake & Kehoe (141, 142) using different methods. Cremer's method of estimation of triethyl lead was based on the formation of a triethyl lead-dithizone colored complex in a chloroform extract (tetraethyl lead does not form a colored complex with dithizone), and this complex was distinguished from diethyl lead by its absorption spectrum.

In the method of Stevens *et al.* (142) isolation of triethyl lead depended upon extraction of hemogenized liver with pentane, conversion to the benzoate salt, and examination of the isolated material by infrared radiation, melting point, and lead analysis. Crystals obtained by evaporating to dryness, leaching with iso-octane, and further decantation and evaporation, melted within a few degrees of the melting point of triethyl lead benzoate and over 60 degrees below that of diethyl lead benzoate. These results were considered to indicate that an appreciable part of the inhaled tetraethyl lead is covered to the trivalent lead ion, which then persists for a time in the body. This view was confirmed by Cremer, who gave 10 mg. per kg. of triethyl lead chloride intraperitoneally to rats and analyzed the tissues for triethyl lead at intervals between two hours and four days. It was found that the level of this compound remained almost unaltered during this time, so that any subsequent degradation to diethyl or inorganic lead takes place more slowly than the initial conversion to triethyl lead. It was also found that though the amount of triethyl lead was less in the brain than in other tissues, brain slices were metabolically highly sensitive to this compound as well as to tetraethyl lead. Inhibition of oxygen uptake, increase in lactate, and decrease in pyruvate were produced by both, but nearly double the dose of tetraethyl lead was required to cause effects equivalent to those of triethyl lead. Diethyl lead chloride had a much less marked effect, and the metabolic disturbances caused by all the organo-lead compounds differed from that following administration of lead acetate in that the latter caused no alteration in the oxygen consumption and only a small change in the lactate and pyruvate levels. The conversion of tetraethyl lead to triethyl lead appears to be brought about by enzymes in the liver. The exact location of the principles involved is not known, but, according to Cremer, 94.5 per cent of the activity of the whole hemogenate is found in the supernatant fluid which contains the microsomes and soluble material and

which remains after large particles, including nuclei and mitochondria, have been removed.

That the toxicity of tetraethyl lead is mainly attributable to its decomposition product, triethyl lead, and not to the diethyl compound, was suggested by the fact that rats receiving either tetra- or triethyl lead developed identical signs of poisoning—excitability, tremors, and convulsions—whereas those given diethyl lead showed only slight initial uneasiness and loss of appetite. Inhibition of cholinesterase has also been suggested as an important factor in the mechanism of poisoning by tetraethyl lead. Smusin (135) found that a dose of 1.07 gm. per kg. produced a severe depression of true cholinesterase in the cerebral cortex, medulla, and muscle tissue of mice, reaching its lowest level in four to eight hours. There was a much less marked depression of the pseudo-cholinesterase of these tissues. Preliminary treatment of the tissues with acetone in the cold had no obvious effect on the cholinesterase level, a fact which it is suggested, provides indirect evidence of the ability of tetraethyl lead to penetrate into every tissue, including lipoid.

Chelation treatment, effective with inorganic lead poisoning, does not appear to be so with tetraethyl lead. Kitzmiller, Cholak & Kehoe (77) did find that intravenous administration of calcium versenate caused a varying increase in the urinary excretion of lead but not in the blood level. In view of the facts that triethyl lead does not combine with either BAL or calcium versenate and that its ion, to quote Stevens *et al.* (142), "is a monovalent electrolyte that probably cannot be chelated," it is unlikely that chelating agents would prove effective as a therapeutic measure.

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REVIEW OF REVIEWS[1]

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Documentation of scientific material is now a major problem in the great nations; no longer can it be handled by a single person or private group. The American Medical Association stopped publishing *The Quarterly Cumulative Index Medicus,* and the National Library of Medicine assumed responsibility for indexing part of the flood of medical reports in the mechanized monthly, *Index Medicus* (New Series, Volume 1, No. 1, January, 1960). Time was (about 1865) when one man, John Shaw Billings, could confidently undertake to catalogue and index the whole of past and current medical literatue. That he did so successfully for so long is testimony to his own skill and persistence, and also to the relative trickle of medical publications during his time. It now takes many experts to cope with the flood. How can we find what is known and where?

In pharmacology the very large number of chemicals studied for their interactions with living material greatly complicates the problem of documentary control. In 1959 over 350 new drugs and drug mixtures were released for prescription use by the Food and Drug Administration of the United States Department of Health, Education, and Welfare. How can we digest the information available on them? The new *Index Medicus* may aid by indexing pharmacological information applicable to the health professions, but it does not index all reports in this field. Abstract services are also incomplete and highly selective, although *Chemical Abstracts, Biological Abstracts,* and *Excerpta Medica* fairly well cover the significant pharmacological reports. Patent literature on new drugs is bewildering, but is covered in part only in *Chemical Abstracts.*

There is now growing interest in critical reviews of pharmacological information, especially that dealing with drug types and mechanisms of action. *Physiological Reviews* started this trend in 1920. *Pharmacological Reviews* has well provided critical reviews since 1949. *The Journal of Pharmacy and Pharmacology* also offers useful reviews of pharmacological reports. Sometimes valuable reviews of pharmacological interest appear in *Science, Endeavour, The American Scientist,* and even in the more popular *Scientific American.* Similar reviews are to be found in *Artzneimittelforschung, Experientia,* and in clinical periodicals such as *The Journal of the American Medical Association.*

A significant indication of the growing importance of the reviewing effort is the increasing number of, and attendance at symposia at which recognized pharmacologists in a special field report and criticize each other's findings. The New York Academy of Sciences has sponsored many

[1] The survey of the literature pertaining to this review was concluded in July, 1960, and covers, generally, the preceding three years.

such symposia of pharmacological interest. A helpful new development is the publication of such symposia with critical discussion. This has gone further to the publication of volumes covering reports given at special conferences called by interested drug manufacturers on some particular new drug. Here can be brought together in one place all the available pharmacological information on the new drug, together with a critical analysis of its possible clinical applications or usefulness.

Publication of reviews, symposia, and conference proceedings is the best method now current for effective interscience communication. It is pertinent to suggest that, as this communicative activity increases, library workers in the experimental sciences may acquire as significant a status as the contributing laboratory workers themselves.

In one sense, textbooks in pharmacology comprise more or less comprehensive reviews of the whole field as it appeared at the date of issue. More detailed are the specific reviews in *Handbuch der Experimentallen Pharmacokolgie.* Beckman's *Yearbook of Drug Therapy* (*7*) is an excellent critical survey.

Special comment is deserved by *Pharmacological Reviews.* Started hesitatingly in 1949, after having been suggested more than a decade earlier, it now offers orientation in major areas of current pharmacological interest. Material in *Pharmacological Reviews* is selective, and invitations are sent to those considered to be competent to prepare the proposed reviews. Invitation reviews reflect the compromised interests, biases, and prejudices of the editors. In all of this, as in editing any scientific journal, there is danger of an insidious growth of an acceptable and conventional canon. This could be a threat to scientific freedom as the power of fund-granting agencies increases. *Pharmacological Reviews,* thanks to the scientific integrity of its editors, seems to have avoided this hazard so far. Now *Annual Review of Pharmacology* comes to help pharmacologists keep abreast of important advances in their science. The value of this extensive review effort depends on the extent of critical appraisal of data and conclusions as shown by individual reviewers.

Reviews of Basic Pharmacological Problems

The most general review of pharmacology's current status as a science is the effort by Leake (52) indicating the five historically characteristic scientific problems with which pharmacology deals and which are peculiar to it as an independent discipline: (*a*) dose-effect relationships; (*b*) the relation of absorption and distribution of chemicals in living material to their metabolism in and removal from this living milieu; (*c*) localization of the site of action of chemicals on living material; (*d*) mechanisms of drug action; and (*e*) relations between chemical constitution and biological action. This review included a consideration of the growing applications of pharmacology not only in the health professions, but also in agriculture, sociology, warfare, and law. It also contained a historical bibliography.

A current pharmacological problem of increasing interest concerns the

long known, but little studied placebo effect. A comprehensive and detailed review, which includes 1063 references, has been made by Beecher (8). This review is in two parts: the first part deals with the measurement of pain as a prototype for the study of subjective responses, and the second part considers the quantitative study of the effects of drugs on various subjective states. As Beecher suggests, studies in these fields are basic to the behavioral sciences. It might be pertinent then to use projective psychological tests, now so skillfully quantified by Harrower (40), as an adjunct to methods used to investigate subjective and emotional factors in the effects of drugs.

Beecher had previously reviewed the problem of the measurement of pain (9). Wolf (95) has more recently analyzed the pharmacology of placebos, detailing the technique and pitfalls of "double-blind" testing, and attempting correlations with interpersonal relations between subjects and service personnel, emotional and physical conditioning, and the general ecological milieu.

Waife & Shapiro (86) have edited a comprehensive survey of factors involved in the clinical evaluation of new drugs which considers general pharmacological principles, experimental design, the appraisal of subjective responses, the ethics and methods of human experimentation, the training of the investigator, clinical trials in practice, illustrative material from various medical specialties, and publication. Much of this is now stereotyped conventionality, but it remains basic for successful clinical pharmacology. The general principles were outlined years ago. Many remain honored in the breach, among them are (*a*) the ratio of the rates of absorption and distribution of the drug through the body to the rates of its disappearance from the body, (*b*) time-concentration relations, and (*c*) elementary dose-effect relations, especially between doses necessary for effectiveness and those which may cause unwanted toxic reactions in sensitive patients.

Absorption, Distribution, Metabolism, and Excretion of Drugs

An interesting review offering suggestions about placental transmission of chemicals has been made by Hagerman & Villee (36) in considering the transport function of the placenta. Renal tubular excretion of organic bases has been well reviewed by Peters (65). Active transport is involved with strong bases, whereas excretion of weaker bases varies inversely with urinary pH. Sperba (79) analyzed the factors concerned in the secretion of organic anions in the formation of urine and bile. Tobian (82) surveyed the interrelationships of electrolytes, juxtaglomerular cells, and hypertension.

A significant recent review on drug metabolism is that made by Young & Maw (96) on the metabolism of sulphur compounds. From a detailed, biochemical discussion, this well-organized little volume goes on to a consideration of various sulphur-oriented drugs. In the symposium on catechol amines, edited by Krayer (50), there are several short reviews on their metabolism and excretion.

Drugs Used in Diagnosis

It is not always appreciated that chemical agents which are used as diagnostic aids are really drugs and, thus, may have unexpected effects on living material when attention is focused merely on their diagnostic use. A symposium on radiopaque diagnostic agents has been held under the auspices of the New York Academy of Sciences, and the proceedings of this symposium have been edited by Poppell (69). Most radiopaque diagnostic agents contain iodine, which by its density affords radiographic contrast. It is the iodine in these compounds which contributes so greatly to the expense involved, and it is also the iodine that may contribute to the toxic effects.

An excellent review of radiopaque diagnostic agents is given by Knoefel (49). Citing over 400 references, this covers much relevant data on physical and optical factors in the interrelations of chemical compounds and x-rays with detail on various types of compounds in regard to visualizing the alimentary tract, the biliary system, the urinary system, the circulatory and respiratory systems, the spinal-subarachnoid space, the genital tract, and the reticulo-endothelial system. Miscellaneous procedures are indicated and toxicity is examined with special care.

Chemotherapy

Perennial interest in amebiasis is reflected in a review by Anderson of newer drugs for handling the disease (2). This well supplements his review of a decade ago. Newer agents are glycobiarsol [N-(2,4-dichloro benzyl, N-2-hydroxy ethyl)-dichloracetanide], a related compound [5,6-quinone-4,7-phenanthroline], and dipiperonyl derivatives. Pharmacological details are not readily available on many of these agents: judgment seems to be made chiefly on empirical clinical observations. Even here there seems to be little data on comparisons with such still widely used drugs as carbarsone and vioform which were introduced three decades ago.

That there is also continued interest in antihelmintics is clear from the survey by Brown of their actions and uses (16). Although effective drugs now seem to be available for all worm infestations, it is to be expected that efforts will be made to find even safer and more active antihelmintics.

Although a tremendous screening program is under way on antitumor drugs, no review of what is being accomplished has appeared. This is not surprising: the "crash" effort at the chemotherapy of cancer is as wild as hunting for honey in a hayfield; the methodology is about the same as Ehrlich's 60 years ago. Nevertheless, Mandel (56) has well reviewed the anabolic and catabolic action of certain antitumor compounds, such as analogues of purine, pyrimidine, and coenzymes, and derivatives of chloroethyl and ethyleneimine, urethane, podophyllin, and colchicine. An extremely comprehensive and detailed review on fundamental aspects of normal and malignant growth has recently been edited by Nowinski (63). This review is basic for any rational program directed toward effective chemotherapy of cancer.

An excellent general review of principles and methods of chemotherapy

has been made by McIlwain (58) in application to diseases of the central nervous system. In addition to the conventional aspect of the chemotherapy of specific infectious diseases, this discussion considers broader applications in noninfectious diseases. Chemotherapeutic considerations go a bit beyond conventional pharmacological and toxicological detail: they probe interactions between drugs and living material in regard to specific pathological factors which are present, and toward the correction of which the chemotherapy effort specifically aims. This volume thus offers background theory for neuro- and psychopharmacology.

Shaw has written an important review on caries-inhibiting agents (75). The paper emphasizes dietary restriction on fermentable carbohydrates and fluoridation of water. Dentrifices containing antibiotics, urea, and ammonium phosphate are not satisfactory for prevention of caries.

A helpful symposium review on the chemical control of plant and animal pests, which deals with a type of ecological chemotherapy, has been edited by Reitz (72).

The detailed analysis by Long (55) remains as the most satisfactory and critical review of the chemotherapy of tuberculosis. This considers sulfones, *p*-aminosalicylic acid, thiosemicarbazones, isoniazide, pyrazinamide, cortisone, hormones, and antibiotics.

There is continued vigorous exploitation of antibiotics—some of it highly questionable, as public hearings have shown. Three recent symposia are especially interesting: one, edited by Goldberg (33), deals with chemistry and nonmedical uses; a second, edited by Subrahmanyan (80), gives much information on antibiotics used in India; the third, edited by Waksman (87), deals with neomycin. A brief review of streptomycin and dihydrostreptomycin was prepared by Weinstein & Ehrenkrantz (91).

Toxicology

Several interesting toxicological reviews have recently appeared. Curry briefly surveyed the application of new analytical techniques to the identification and quantitative estimation of poisons (21). Barnes & Stoner offer the first systematic survey of the toxicology of tin compounds (5). In a detailed analysis of the biochemistry of animal poisons, Kaiser & Miehl give much valuable toxicological data (46). This volume is helpful in its survey of the mechanisms of action of the many poisonous substances elaborated by animals. The Wielands have prepared a definitive survey of the complex chemistry and toxicology of the toxins of *Amanita phalloides,* the poisonous toadstool (93).

The Actions of Drugs on Particular Systems

Pharmacological orientation toward mammalian physiological systems continues. Currently it is focused chiefly on the central nervous system; however, circulation remains interesting enough to draw several recent reviews. Aviado (3) suveys the pharmacology of the pulmonary circulation, a study long overdue. Price (71) analyzes the ways that circulatory homeo-

stasis is maintained under the stresses of general anesthesia, and how it may fail with particular substances. The complex actions of many different types of drugs on cerebral circulation are surveyed by Sokoloff (78).

Hypotensive drugs are reviewed in a symposium edited by Harington (39). This deals with veratrum alkaloids, amidine derivatives, iminazolines, reserpines, ganglionic blocking agents including mono- and bis-onium salts, and haloalkylamines. The chemistry and pharmacology of hypotensive drugs is discussed in relation to their clinical use. Hormones in relation to atherosclerosis are surveyed in a symposium edited by Pincus (66).

An important recent review is Baker's on the effects of drugs on the fetus (4). This stresses the paucity of precise information on the potential dangers to feti from various types of drugs given to pregnant females. The muscle-relaxing pharmacological action of quaternary ammonium compounds, both depolarizing and nondepolarizing, is reviewed by Foldes (29) in a summary considering their use in anesthesiology.

Enzymes and Enzyme Inhibitors

Greenberg & Harper (35) have edited a comprehensive review of enzymes in health and disease that covers chemical properties and mechanisms of enzyme action, energy transformations, relations to hormones, inborn metabolic errors, lipid metabolism, enzyme aspects of neuronal, mental, and eye disease, effects of drugs on enzyme systems, tumor enzymology, and the clinical use of enzymes as drugs. Here are discussed trypsins, deoxyribonuclease, collagenase, hyaluronidase, thromboplastin, fibrinolysins, and penicillinase. There is even a discussion of the use of enzymes as diagnostic drugs. The volume is well documented but not indexed.

Clinical aspects of enzymology and the use of various enzyme preparations were reviewed by Martin (57). He also edited a symposium on proteolytic enzymes and their clinical application. This included information on plasmin, trypsins, anti-inflammatory enzymes, and use of enzymes in veterinary medicine.

Of increasing pharmacologic interest are the heparin derivatives. Robinson & French have neatly reviewed the subject of heparin, fat transportation, and the clearing factor lipase (73).

The general field of anticholinesterases remains very active, but many aspects of it are unavailable for review, since they relate to chemical warfare. Holmstedt has fully reviewed the available material on the pharmacology of organophosphorus choline esterase inhibitors (42). Zeller edited a survey of amine oxidase inhibitors (97). Fundamental to an understanding of the relation of metals to enzyme action is the symposium edited by Crook (20). Coenzymes derived from water-soluble vitamins were reviewed by Brown (15).

Diuretics

Several summaries of the current status of diuretics have recently appeared. Buchborn & Bock edited an international symposium on diuresis and diuretics (18). This was duplicated in large part by a more extensive

symposium discussion edited by Moyer & Fuchs (62). The latter deals chiefly with mechanisms of edema and their management, whereas the former is concerned mainly with mechanisms of diuresis. Both give much pharmacological detail. Symposia treatment is well suited to this co-ordination of physiological and pharmacological considerations.

With greater emphasis on physiological aspects on pharmacological, Pitts (67) nevertheless offers a keen discussion for rational diuretic therapy. Handley & Moyer, however, place prime emphasis on the character and mode of action of diuretic drugs (38). All discuss the diuretic aspects of carbonic anhydride inhibition, xanthines, mercurials, triazine and thiazide compounds, antialdosterones, and steroids.

A special symposium on aldosterone antagonists was held in Chicago in 1958, and Bartter recently edited the discussion (6). It dealt with spirolactones which oppose the action of aldosterone and other sodium-retaining steroids. This occurs at dosages which have no other physiologic effect. The most effective antialdosterone is called spironolactone (Aldactone).

Oral Antidiabetic Drugs

The development of orally effective compounds for the treatment of diabetes mellitus has been reviewed by Duncan & Baird (25). This review considers the pharmacology, toxicity, mechanisms of action, and clinical use of various sulphonylureas and guanadine derivatives. Many modifications of compounds of this type have been prepared, and clinical evaluation progresses slowly. Toxicity has been observed in continued use of some of these agents, and various kinds of untoward effects have been described. The action of these types of compounds seems to be at cellular levels. The aryl sulfonamides directly stimulate beta cells in the pancreas to put insulin into the blood, and there is some evidence that they may increase the number of beta cells. Blood assay of insulin, which could be used to evaluate the effects of aryl sulfonamides, has been reviewed by Vallance-Owen & Wright (84).

The chief aryl sulfonamides useful as antidiabetic drugs were developed by Loubatieres in Montpelier. They are 1-butyl-3-sulfonilylurea (carbutamide), 1-butyl-3-p-tolysulfonylurea (tolbutamide), and 1-propyl-3-(p-chlorobenzenesulfonyl) urea (chlorpropamide). The latter is effective in low dosage and is long acting. A special symposium dealing with this drug was edited by Goldner (34).

Aromatic Alkylamines

An outstanding symposium on the pharmacology of catechol amines was edited for publication by Krayer (50). This covers information on their metabolism, and the consequences thereof, with discussion on all aspects of their biological activity. The symposium was a pertinent reflection of pharmacological interest in the pathochemistry of mental disorder, and was directed toward ways for correction of chemopathology.

The amphetamines were reviewed by Leake (51), with detail on ac-

tions, toxicity, and clinical use, and with comment on sociological considerations in regard to their possible abuse. This review deals chiefly with amphetamine, dextro-amphetamine, and methamphetamine. It does not include diethylproprion, an amphetamine derivative used for depressing appetite.

Lewis edited an international symposium on 5-hydroxytryptamine, in which its biochemistry, physiology, pharmacology, and possible clinical significance were considered (54). Von Euler has reviewed the actions of epinephrine and norepinephrine, and their use in humans (85).

Related to the pharmacology of amines is the increasingly significant investigation of amine oxidase inhibitors. These compounds resulted from detailed studies by Brodie and his companions at the National Institutes of Health on the enzymatic metabolism of various amines. Most of the monoamine oxidases are amino acid and fatty acid hydrazides, typified by iproniazid. However, many conjugated types of hydrazides exhibit amine oxidase inhibition. The New York Academy of Sciences symposium on amine oxidase inhibitors, edited by Zeller (97), gives details on the biochemorphology, general pharmacology, toxicity, and clinical evaluation of amine oxidase inhibitors as "psychic energizers."

Specifically dealing with the action of various drugs on the secretion, distribution, and excretion of epinephrine and norepinephrine is the excellent summary prepared by de Schaepdryver (22). He analyzes the influence of such drugs as ganglionic stimulants, histamine, morphine, caffeine, reserpine, insulin, nicotine, and iproniazid.

Specific Drugs

Many symposia and reviews have recently been published on specific drugs. A stimulating symposium was edited by Berger on non-narcotic drugs for relief of pain (11). This included much data on proposed mechanisms of action, together with psychological factors of intensification or reduction of the sense of pain. He dealt with such drugs as carisoprodol, zoxazolamine, phenyramidol, and phenylbutazone. Another symposium dealing solely with carisoprodol was edited by Miller (59). As in the case of similar symposia designed to assemble together in one place available information on a new drug, it is long on clinical testimony and short on pharmacological science. This comment also applies to the two volumes published as reviews on trifluoperazine, a new phenothiazine "tranquilizer," one edited by Brill (14), the supplementary volume by Moyer (61). Nevertheless, these volumes do save a great deal of searching through scattered periodicals to find the separate reports. Further, they save space in both scientific and clinical periodicals, and they save much trouble in documentation and retrieval. There is much to commend the publication as a symposium of the available information on a new drug—provided always that the survey is critical and that unfavorable observations are emphasized and discussed as fully as favorable ones. This is the case in the symposium on meprobamate, edited by Berger (10).

Interesting in this regard is the symposium edited by Brust (17) on triparanol, a nonfeminizing estrogenic agent which blocks the biosynthesis of cholesterol. Here unfavorable opinion was expressed not only on the theory that cholesterol is responsible for many cardiovascular disorders, but also on the possible untoward effects of increasing desmosterol concentration. Nevertheless, empirical clinical opinion indicates potential usefulness of the drug in coronary disorder.

Digitalis has recently been reviewed in a volume edited by Dimond (23) This includes much pharmacological data on related cardiac glycosides. A more systematic pharmacological review on the cellular basis of cardiac glycoside action was made by Hajdu & Leonard (37). Much stimulation for fundamental pharmacological research may be found here. It is rather peculiar that pharmacologists have not often been attracted to studying the action of drugs at a cellular level using the tissue-culture method, with phase-contrast microscopy and time-lapse movies, as developed so well by Pomerat (68).

A very extensive review of curare and curare-like agents has been made by Bovet and associates (12). This is a detailed consideration of the chemistry and biochemorphology of these compounds, as well as a discussion of their mechanisms of action and toxicity.

The pharmacology of plant phenols has been systematically reviewed by Fairbairn (26). Dealing primarily with plant estrogens, flavonones, and photodynamic compounds, it also considers various types of anthraquinones. These agents are discussed from the standpoint of their fate in the body and their potential toxicity. Porter has edited an important symposium on the biological activity of growth substances (70). A comprehensive review prepared by Gilbert (32), recently appeared on the mechanisms of the hemodynamic effects of endotoxin. One may question whether or not endotoxin is a matter for pharmacological consideration, but certainly endotoxin does have significant effects on living material, and as its chemical constitution is more clearly established, it may suggest related and competing pharmacological agents of clinical usefulness.

Chelating agents are becoming increasingly important in pharmacological application, particularly in connection with the hastening of the excretion of radioactive material. A general, comprehensive symposium on metal binding in medicine has been edited by Seven & Johnson (74). This covers the pharmacology and toxicity of various types of chelating agents and discusses particularly the value and use of disodium ethylene diamine tetraacetate.

The management of various anemias with iron compounds remains clinically important. The general pharmacological and clinical aspects of iron in clinical medicine has been reviewed in a symposium edited by Wallerstein & Mettier (88). The symposium contains much helpful information on the absorption and metabolism of iron.

Local anesthesia has long been a favorite field for synthetic studies by organic chemists and for routine pharmacological screening. The mode of

action of local anesthetics is more complex than is ordinarily considered to be the case. This matter has now been well surveyed by Watson (89).

In relation to the extraordinary population explosion which is disturbing the whole world, there is increasing interest in various types of antifertility agents. Many of these are compounds which interfere with ovulation and are progesterone competitors. Experimentation has only recently begun on other types of compounds that may alter fertility by other mechanisms. The field of antifertility substances has been reviewed by Jackson (43).

Neuro- and Psychopharmacology

As a result of the initial excitement beginning in 1953 over the effectiveness of Rauwolfia compounds and chlorpromazine in calming disturbed mental patients, a most extraordinary recent scientific development has been the tremendous interest in neuro- and psychopharmacology. The most extensive and systematic reviews in neuropharmacology have been the Macy Conferences edited by Abramson (1). Five such conferences, from 1954 to 1959, have been held, with published transactions. These conferences on neurophysiology covered most of the important aspects of the current activity in the field. The value of the Macy Conferences consists largely in their critical nature. The free and open discussion helps to clarify issues as they arise and clearly points out the inadequacies of data for anything more than tentative conclusions.

The problem of evaluating studies in psychopharmacology was carefully reviewed in a special symposium held under the auspices of the National Research Council, and edited by Cole & Gerard (19). This was a discussion of primary importance for the establishment of basic principles for judgment in studies on psychopharmacology.

Many special symposia, often of an international nature, have been held that deal with various aspects of psychopharmacology. Featherstone & Simon edited an interesting conference using a pharmacologic approach to the study of the mind (27). A similar symposium held in Milan to deal with psychotropic drugs, was edited by Garattini & Ghetti (30). Another symposium devoted chiefly to tranquilizing drugs was edited by Himwich (41). Kline edited a special symposium dealing with psychopharmacology (47), and another international symposium held in Zurich which considered psychopharmacology frontiers (48). Both of these symposia brought together a large amount of new material concerned with the psychological and behavioral effects of various types of drugs.

An international symposium dealing only with a special amine oxidase inhibitor, nialamide, was held in Lisbon, and the proceedings were edited by Leitao (53). A considerable symposium on psychopharmacology, with 41 contributors, was edited by Pennes (64). This considers basic mechanisms, clinical applications, the serotonin hypothesis of psychomimetic action, electrophysiological analyses of drug action, the effects of indoles on the central nervous system, and psychoanalytical aspects of tranquilizing drugs. Another general symposium dealing with neuropsychopharmacology and

held in Nuremberg has been edited by Flügel (28). This was largely a review of current German work relating to psychopharmacology.

An interesting review of certain aspects of neuropharmacology was made by Gibbs (31). This relates to amines in regard to brain function and behavior, and then considers the use of ACTH in hypsarhythmia, the lightning-fast major seizures which occur in children.

A number of short reviews on the pharmacology of psychotropic drugs have appeared. Jacobson reported on the comparative pharmacology of certain psychotropic compounds (44). Miller & Berry (60) have reviewed the motivational effects of drugs, considering methods which illustrate general problems in psychopharmacology. Sidman made a brief but helpful review on behavioral pharmacology (76). The action of drugs on the cerebral circulation was covered by Sokoloff (78), and Weinstein has given an interesting survey of language in respect to psychopharmacology (90).

Zeller has edited a general symposium and conference on amine oxidase inhibitors in relation to their use as antidepressants in psychotic states (97). Brill (14) and Moyer (61) have edited review symposia on the phenothiazine "tranquilizer," trifluoperazine.

The most satisfactory, detailed, and critical review on the relation of psychiatry to pharmacology has been made by Wikler (94). This review covers 889 references and is specially indexed. It considers specifically the effects of various drugs on human behavior, with comment on theories and mechanisms. Wikler's review will remain a standard of excellence for detailed critical analysis of drug action on the central nervous system.

Among other reviews dealing with neuro- and psychopharmacology to appear recently is the comprehensive volume edited by Braceland (13). A brief orienting review of psychotherapeutic drugs is offered by Welsh (92). Useful for quick reference to names, formulae, doses, and untoward effects is the tabulated summary of psychopharmacological agents used in psychiatry as prepared by the Psychopharmacology Service Center of the U.S. Department of Health, Education, and Welfare (83).

Continuing Drug Reviews

In addition to the *Annual Review of Pharmacology*, there now appears *Progress in Drug Research* (45), edited by Jucker of Basel. The first two volumes (1959, 1960) contain significant reviews of current problems of pharmacological research.

A special series of continuing reviews on medicinal chemistry is under the editorial direction of Blicke & Cox for the American Chemical Society. These are prepared with tabulated detail, extensive bibliographies, and excellent indices. A recent example is the comprehensive survey of barbituric acid hypnotics by Doran, with 230 tables and 1204 references (24). Another series has been sponsored by the Institute for the Study of Analgesic and Sedative Drugs. The fourth in this series is Smith's well-prepared review of acetophenetidin, with 529 references (77).

Annual surveys of new drugs may become increasingly useful as the

number of new drugs increases each year. Tice has made such a listing for 1959 (81).

Prospect

Critical reviews of pharmacologic information are becoming increasingly important in digesting the plethora of pharmacological and toxicological data and opinion accumulating on the myriad of new drugs now flooding us. Such reviews are indispensable to the rational clinical use of new drugs. It is an indication of the growing responsibility of drug makers that they are supporting symposia directed toward the preparation of such reviews. The publication of symposia on new drugs in a single volume helps greatly in saving laborious literature searching through many periodicals in order to obtain the information available. The value of these symposia volumes will continue to depend on their critical attitude, especially in regard to toxic effects.

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